Emergence and Development of Red Sorrel (*Rumex acetosella*) and Lowbush Blueberry (*Vaccinium angustifolium*) Ramets in Lowbush Blueberry Fields

By

Scott Neil White

A Thesis

presented to

The University of Guelph

In partial fulfilment of requirements

for the degree of

Doctor of Philosophy

in

Plant Agriculture

Guelph, Ontario, Canada

© Scott Neil White, December, 2013
Field studies were established to evaluate the suitability of growing degree day (GDD, \( T_{\text{base}}=0 \, ^{\circ}\text{C} \)) models for predicting emergence and development of lowbush blueberry and red sorrel ramets and to conduct a demographic study of red sorrel in lowbush blueberry. Model predictions for initiation of lowbush blueberry ramet emergence, tip dieback, and flowering were 243, 692, and 389 GDD, respectively. Peak lowbush blueberry ramet emergence and tip dieback occurred around 928 and 1626 GDD, respectively. Peak blueberry bloom was observed between 552 and 565 GDD. Model prediction for the initiation of red sorrel ramet emergence was 92 GDD, and 10, 50, 90, and 95% emergence were predicted to occur at 279, 1322, 2536, and 2696 GDD, respectively. Red sorrel ramets therefore initiate emergence earlier than lowbush blueberry, and continue to emerge during important crop developmental processes in the non-bearing year. Model prediction for the initiation of red sorrel flowering was 289 GDD, and 10, 50, 90, and 95% flowering were predicted to occur at 376, 545, 877 and 1336 GDD, respectively. Red sorrel ramets therefore flowered during blueberry bloom and may interfere with crop
pollination. Red sorrel ramet emergence was season-long but ramets suffered higher mortality during the bearing year. Red sorrel ramet mortality was higher in bare soil patches than within blueberry clones. A distinct overwintering red sorrel ramet population was identified and constituted the majority (>70%) of the population of flowering ramets. Seasonal recruitment of new red sorrel seedlings occurred and indicates regular recruitment of new genets into established populations. Results of field studies initiated a series of greenhouse and growth chamber studies to investigate the roles of vernalization and photoperiod on red sorrel ramet development. Red sorrel ramets had an obligate requirement for vernalization and only flowered when transferred to long days after cold treatment. Ramets required vernalization for at least 10 weeks to induce flowering. Pre and post-vernalization ramet removal reduced flowering ramet density under both field and controlled conditions. Exposure of ramets to decreasing photoperiod prior to vernalization increased flowering frequency in one experiment and indicates a potential role of pre-vernalization stimuli in regulating ramet flowering.
ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my co-advisors, Drs. Nathan Boyd and Rene Van Acker. Nathan, I have learned more from you, as both an employee and a student, than I could have ever imagined. You are a great teacher, mentor, and friend. Thank you so much for everything you have done for me. Thank you Rene for your guidance and genuine interest in the research project; your input on early proposals and chapters has been invaluable. I sincerely thank both of you for supporting my ideas and trusting in my ability. I also thank you both for supporting my decision to divide my time between Nova Scotia and Guelph – this made things difficult at times but having a great advisor in each location made a world of difference.

I would also like to acknowledge the contributions of my advisory committee, Drs. Clarence Swanton and Steven Newmaster. Your feedback on the thesis has been extremely valuable and I thank you for continually challenging my ideas and pushing me to work harder. I would also like to thank Dr. Swanton for welcoming me into the weeds lab during my time at Guelph. I really felt as though I were a part of two weeds labs during my graduate studies.

I would like to acknowledge all of the students and technical staff in Dr. Boyd’s research program in Nova Scotia. Their assistance was essential to the completion of various aspects of the field work. I would specifically like to acknowledge Karen Kennedy, Angela Hughes and Megan Mceachern for their help prior to my arrival back in Nova Scotia after the first winter in Guelph, and Emily Clegg and Swati for assistance during the last field season.

I would like to acknowledge the assistance of Karen Smith and Fred Fergus at the Dalhousie Agriculture Campus for help finding greenhouse and growth facility space. These resources are limited at this campus, and your help was crucial to the completion of the various
flowering experiments conducted. To Fred and Craig in the IT department, thank you for helping with my unusual requirement for two computer CPU fans, a CD-ROM drive and a computer power supply. They worked perfectly! I would also like to thank Doug MacDonald at the Dalhousie Agriculture Campus for helping me establish a cold room for the vernalization treatments. It was a delicate balance between storing potatoes and vernalizing red sorrel in the same room, and I simply would not have been able to get it done without Doug. I also acknowledge the statistical advice of Dr. Tess Astatkie.

Funding for much of the research was provided in part by an NSERC IPS Scholarship supported by DuPont Canada. I sincerely thank Bill Summers for his support, and Jenney Van De Kamer for all the help in setting up the scholarship. Funding for equipment requirements was provided by the Canadian Foundation for Innovation. I am also grateful to the University of Guelph for various bursaries and awards received during my graduate work. I would also like to acknowledge the late Beth Livingstone. Beth was one of the first people I met in Guelph, and her genuine kindness and welcoming attitude made me feel right at home.

I thank Doug Wyllie and Jason Stuart at Bragg Lumber Company and Wendell Purdy of Purdy Resources for allowing my research to be conducted on their blueberry land. The accommodation of researchers by growers is essential for advancement of research in lowbush blueberry and it is acknowledged.

Last, but certainly not least, I would like to thank my family and friends for all of their support during this process. The friends I have made in both Guelph and Nova Scotia have been great. Thank you to Maria and your parents and Linda and Randy – you were both wonderfully welcoming during my stays in Guelph. Thanks Jeff for the support during the qualifying exam
semester. Thank you Mom and Dad for your unwavering support of my seemingly endless career as a student. To Joanna, I say thank you for your patience, love, and support. In many ways this truly was a team effort and I am forever grateful to you. Finally, I would like to thank Anouk for keeping me company in my home office. Your random visits and decisions to sleep on my stacks of papers or my keyboard were welcome distractions on many a long day.
Table of Contents

List of Tables .................................................................................................................. xii
List of Figures .................................................................................................................. xvii
List of Abbreviations ...................................................................................................... xxi

Chapter 1: General Introduction and Literature Review .................................................. 1
  1.1. Lowbush Blueberry Crop Value, Production, and Potential for Predictive Models .... 1
  1.2. Lowbush Blueberry Emergence and Development during the Two-Year Production Cycle ................................................................................................................. 4
  1.3. General Weed Situation, Status and Biology of Rumex acetosella in Lowbush Blueberry Fields in Nova Scotia .................................................................................. 9
  1.4. Improving the Current Knowledge of Rumex acetosella Ramet Dynamics with a Demographic Approach ................................................................................................. 13
  1.5. Improving the Current Knowledge of Developmental Segregation in Established Rumex acetosella Ramet Populations with a Demographic Approach ................................................. 17
  1.6. Role of Seedling Recruitment in the Maintenance of Clonal Plant Populations ........ 19
  1.7. Research Objectives .................................................................................................. 20

Chapter 2: Emergence and Development of Wild Blueberry (Vaccinium angustifolium Ait.) Ramets in Nova Scotia ................................................................. 22
  2.1. Abstract .................................................................................................................... 23
  2.2. Introduction .............................................................................................................. 24
  2.3. Materials and Methods ........................................................................................... 28
      2.3.1. Site Selection .................................................................................................... 28
      2.3.2. Weather Data .................................................................................................. 28
      2.3.3. Emergence and Development Data ................................................................. 31
      2.3.4. Development of Thermal Models .................................................................... 34
      2.3.5. Assessing Fit and Validation of Thermal Models .............................................. 36
  2.4. Results ..................................................................................................................... 37
      2.4.1. General Trends in Emergence, Tip Dieback, and Flowering ......................... 37
      2.4.2. Development of Thermal Models .................................................................... 39
      2.4.3. Model Validation ............................................................................................. 39
  2.5. Discussion ............................................................................................................... 45
2.6. Conclusions .......................................................................................................................... 51

Chapter 3 : A demographic study of red sorrel (*Rumex acetosella* L.) in lowbush blueberry fields in Nova Scotia ........................................................................................................... 53

3.1. Abstract .................................................................................................................................. 53
3.2. Introduction .............................................................................................................................. 55
3.3. Materials and Methods .......................................................................................................... 58
  3.3.1. Description of Study Sites and Quadrat Placement ......................................................... 58
  3.3.2. Red Sorrel Ramet and Seedling Dynamics ....................................................................... 60
  3.3.3. Red Sorrel Root Depth ....................................................................................................... 62
  3.3.4. Ramet and Seedling Phenological Development ............................................................. 62
  3.3.5. Ramet Cohort Survival, Age Structure, and Life-Cycle Model ..................................... 63
3.4. Data Analysis ......................................................................................................................... 64
  3.4.1. Red Sorrel Ramet and Seedling Dynamics ....................................................................... 64
  3.4.2. Root Biomass .................................................................................................................... 65
  3.4.3. Ramet Cohort Survival, Age Structure, and Life-Cycle Model ..................................... 65
  3.4.4. Ramet and Seedling Phenology ....................................................................................... 66
3.5. Results .................................................................................................................................... 68
  3.5.1. Red Sorrel Ramet and Seedling Dynamics ....................................................................... 68
  3.5.2. Red Sorrel Root Depth ....................................................................................................... 74
  3.5.3. Ramet and Seedling Phenological Development ............................................................. 75
  3.5.4. Ramet Cohort Survival, Population Age Structure, and Life-Cycle Model .................. 79
3.6. Discussion ............................................................................................................................... 89
3.7. Conclusions ........................................................................................................................... 102

Chapter 4 : Temperature thresholds and degree-day models for red sorrel (*Rumex acetosella* L.) ramet sprouting, emergence, and flowering in lowbush blueberry (*Vaccinium angustifolium* Ait.) fields in Nova Scotia, Canada ......................................................................................... 104

4.1. Abstract .................................................................................................................................. 104
4.2. Introduction .............................................................................................................................. 105
4.3. Materials and Methods .......................................................................................................... 107
  4.3.1. Root Material for Root Sprouting Experiments .............................................................. 107
  4.3.2. Temperature Experiments ............................................................................................... 108
4.3.3. Growing Degree-Day Models to Predict Ramet Emergence and Flowering Under Field Conditions ................................................................. 110

4.4. Results ........................................................................................................ 116

4.4.1. Temperature Experiments ...................................................................... 116

4.4.2. Growing Degree-Day Models to Predict Ramet Emergence and Flowering Under Field Conditions ................................................................. 119

4.5. Discussion .................................................................................................... 121

4.6. Conclusions ................................................................................................. 129

Chapter 5 : Studies on the flowering biology of red sorrel (*Rumex acetosella* L.) in Nova Scotia, Canada ......................................................................... 131

5.1. Abstract ....................................................................................................... 131

5.2. Introduction ................................................................................................ 133

5.3. Materials and Methods ............................................................................. 138

5.3.1. Source of Plant Material ......................................................................... 138

5.3.2. Growing Conditions and General Plant Maintenance ........................... 138

5.3.3. Plant Acclimation and Vernalization ......................................................... 139

5.3.4. General Data Collection and Flowering Assessment .............................. 140

5.3.5. Experiment 1 - Effect of Vernalization and Photoperiod on Ramets Established from Soil Cores ........................................................................ 141

5.3.6. Experiment 2 - Effect of Vernalization and Photoperiod on Ramets Established from Root Fragments ................................................................. 142

5.3.7. Experiment 3 - Effect of Vernalization Duration on Ramets Established from Root Fragments ........................................................................ 143

5.3.8. Experiment 4 - Effect of Vernalization Duration on Plants Established from Seed . 144

5.3.9. Experiment 5 - Effect of Pre and Post-vernalization Ramet Removal Under Field Conditions .................................................................................. 145

5.3.10. Experiments 6 and 7 - Effect of Pre and Post-vernalization Ramet Removal Under Controlled Conditions ................................................................... 147

5.3.11. Experiment 8 - Effect of Decreasing Photoperiod and Decreasing Temperature Prior to Vernalization ................................................................. 149

5.3.12. Statistical Analysis ................................................................................. 151

5.4. Results ........................................................................................................ 153
Chapter 5: The Influence of Vernalization

5.4.1. Experiment 1 - Effect of Vernalization and Photoperiod on Ramets Established from Soil Cores ................................................................. 153
5.4.2. Experiment 2 - Effect of Vernalization and Photoperiod on Ramets Established from Root Fragments ................................................................. 154
5.4.3. Experiment 3 - Effect of Vernalization Duration on Ramets Established from Root Fragments ................................................................. 154
5.4.4. Experiment 4 - Effect of Vernalization Duration on Plants Established from Seed ................................................................. 160
5.4.5. Experiment 5 - Effect of Pre and Post-vernalization Ramet Removal Under Field Conditions ................................................................. 166
5.4.6. Experiments 6 and 7 - Effect of Pre and Post-vernalization Ramet Removal Under Controlled Conditions ................................................................. 169
5.4.7. Experiment 8 - Effect of Decreasing Photoperiod and Decreasing Temperature Prior to Vernalization ................................................................. 174

5.5. Discussion ...................................................................................................................... 179
5.6. Conclusions .................................................................................................................... 190

Chapter 6: General Discussion and Conclusions .................................................................. 191

6.1. Further Development and Use of Predictive Models for Lowbush Blueberry ............... 191
6.1.1. Summary and Restrictions for Current Models ............................................................. 191
6.1.2. Improving Model Performance .................................................................................... 192
6.1.3. Role of Winter Chilling on Thermal Time to Flowering .................................................. 194
6.1.5. Relating Pest Development to that of the Lowbush Blueberry ....................................... 196

6.2. Role of Demography for Management and Study of Perennial Weeds in Lowbush Blueberry Fields ........................................................................... 201
6.2.1. Key Processes Regulating *Rumex acetosella* Populations and the Role of the Proposed Life-Cycle Model for Developing Management Strategies .................................................. 201
6.2.2. Improving the Proposed Life-Cycle Model for *Rumex acetosella* .................................. 206
6.2.3. Role of the Proposed Life-cycle Model for Managing Herbicide Resistant *Rumex acetosella* Populations in Lowbush Blueberry .......................................................... 208
6.2.4. Use of Demographic Data to Study Carbohydrate Movement in *Rumex acetosella* 209

6.3. Prospects of Studying Vernalization in an Herbaceous Creeping Perennial .......................... 211
6.3.1. Impacts of Vernalization on Seeds and Root Buds ....................................................... 211
6.3.2. Impacts of Juvenility on Vernalization of *Rumex acetosella* ..................................... 213
6.3.3. Timing of Flower Primordia Formation ................................................................. 215
6.3.4. Identification of the Optimum Temperature for Vernalization .............................. 217
6.3.5. Identification of the Optimum Photoperiod for Flowering .................................... 218
6.3.6. Movement of the Floral Stimulus in Rumex acetosella ........................................ 219
6.3.7. Vernalization-Induced Genetic Changes in Rumex acetosella .............................. 220
6.3.8. Understanding the Role of Pre-vernalization Stimuli on Vernalization and Flowering Competency ...................................................................................................................... 221
6.3.9. Role of Ramet Polycarpy in Reproduction of Rumex acetosella ............................ 222
6.4. General Conclusions ................................................................................................ 223

Chapter 7: Literature Cited .......................................................................................... 226

Appendix A: Evaluation of MAT28 for crop tolerance and weed control in wild blueberry .......................................................................................................................... 250

A.1. Introduction ............................................................................................................. 250
A.2. Materials and Methods .......................................................................................... 250
   A.2.1. Data Analysis .................................................................................................... 253
A.3. Results .................................................................................................................... 253
A.4. Summary ................................................................................................................ 259
List of Tables

Table 2.1. Description of study sites used to collect data for calibration and validation of growing degree day models developed for lowbush blueberry in Nova Scotia, Canada.................................................. 30

Table 2.2. Parameter estimates and goodness of fit statistics for proposed Weibull and Gompertz equations describing the relationship between growing degree days (GDD) calculated from air temperature ($T_{base} = 0^\circ C$) and percent cumulative lowbush blueberry ramet emergence, percent cumulative ramets at tip dieback, and percent cumulative flowering ramets in Nova Scotia, Canada.................................................................................................................................................. 40

Table 2.3. Estimated growing degree days (GDD) calculated from air temperature ($T_{base} = 0^\circ C$) to reach 10, 50, 90, and 95 percent cumulative lowbush blueberry ramet emergence, percent cumulative ramets at tip dieback, and percent cumulative flowering ramets in Nova Scotia, Canada.................................................................................................................................................. 41

Table 2.4. Parameter estimates and goodness of fit statistics for the Gaussian model describing the relationship between growing degree days (GDD) calculated from air temperature ($T_{base} = 0^\circ C$) and percent of the total number of open flowers on lowbush blueberry ramets in Nova Scotia, Canada.................................................................................................................................................. 42

Table 2.5. Goodness of fit statistics for validation of proposed Weibull and Gompertz models for predicting the relationship between growing degree days (GDD) calculated from air temperature ($T_{base} = 0^\circ C$) and percent cumulative lowbush blueberry ramet emergence, percent cumulative ramets at tip dieback, and percent cumulative flowering ramets in Nova Scotia, Canada. ............. 43

Table 3.1. Description of study sites used for collection of red sorrel ramet and seedling demographic data in lowbush blueberry fields in Nova Scotia, Canada. .................................................. 59

Table 3.2. Analysis of demographic parameters for red sorrel ramet populations in blueberry and bare soil patches in three commercial lowbush blueberry fields in Nova Scotia, Canada. ............. 70

Table 3.3. Mean production cycle $Ro$ values for red sorrel ramet populations in blueberry and bare soil patches in three lowbush blueberry fields in Nova Scotia, Canada. .................................................. 71

Table 3.4. Mean total density, percent survival, and net population density of red sorrel seedlings emerging in blueberry and bare soil patches in four lowbush blueberry fields in Nova Scotia, Canada................................................................................................................................. 71

Table 3.5. Mean red sorrel root biomass at three core depths in blueberry and bare soil patches in three lowbush blueberry fields in Nova Scotia, Canada. ................................................................................................................................. 75
Table 3.6. Percentage contribution of red sorrel ramet cohorts to the total number of flowering ramets in blueberry patches at three non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

Table 3.7. Percentage contribution of red sorrel ramet cohorts to the total number of flowering ramets in bare soil patches at three non-bearing and three bearing-year lowbush blueberry fields in Nova Scotia, Canada.

Table 3.8. Mean density and test of 50:50 segregation of flowering and vegetative ramets in overwintering red sorrel ramet populations in blueberry and bare soil patches in three commercial lowbush blueberry fields in Nova Scotia, Canada.

Table 3.9. Sex ratio of flowering red sorrel ramets in three non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

Table 3.10. Mean density and percent survival of monthly red sorrel ramet cohorts in blueberry patches at one non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

Table 3.11. Mean density and percent survival of monthly red sorrel ramet cohorts in bare soil patches at one non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

Table 4.1. Description of study sites used to collect data for calibration and validation of growing degree-day models developed for red sorrel ramet emergence and flowering in lowbush blueberry fields in Nova Scotia, Canada.

Table 4.2. Parameter estimates and goodness of fit statistics for the Gaussian equation fit to red sorrel ramet sprouting at constant temperatures of 5, 10, 15, 20, 25, and 35°C.

Table 4.3. Effect of constant temperatures on the mean number of red sorrel ramets per 2cm root fragment.

Table 4.4. Parameter estimates and goodness of fit statistics for calibration of the power and logistic equations fit to red sorrel ramet emergence and ramet flowering, respectively, as a function of GDD (T_{base}=0°C).

Table 4.5. Goodness of fit statistics for validation of proposed GDD (T_{base}=0°C) models for predicting red sorrel ramet emergence and flowering in lowbush blueberry fields in Nova Scotia, Canada.

Table 5.1. Description of study sites used to determine the effects of fall and spring red sorrel ramet removal on ramet dynamics and ramet flowering in lowbush blueberry.
Table 5.2. Weighted-Least-Squares ANOVA of flower frequency in red sorrel ramets established from soil cores and exposed to short, long or increasing photoperiods with or without vernalization

Table 5.3. Mean pre and post-vernalization ramet density and ramet leaf number in various photoperiod treatments with and without vernalization

Table 5.4. Weighted-Least-Squares ANOVA of flower frequency in red sorrel ramets exposed to short, long, or increasing photoperiods with or without vernalization

Table 5.5. Percent of cell packs with flowering ramets, mean flowering ramet leaf number and flower stem height, and mean days to bolting of red sorrel ramets exposed to various combinations of photoperiod and vernalization

Table 5.6. Mean final vegetative and flowering ramet density, ramet leaf number, and flowering ramet survival in cell packs exposed to various photoperiod and vernalization treatments

Table 5.7. Weighted-Least-Squares ANOVA of flower frequency in red sorrel seed plants exposed to different vernalization durations

Table 5.8. Flowering frequency, mean time to seed plant and ramet bolting, mean density of flowering ramets, and mean flowering seed plant leaf and stem number in cell packs exposed to vernalization at 4.5 ± 0.1°C for durations of 0, 5, 10, and 15 weeks

Table 5.9. Final seed plant survival, seed plant leaf number, ramet density, and ramet leaf number in cell packs exposed to vernalization at 4.5 ± 0.1°C for durations of 0, 5, 10, and 15 weeks

Table 5.10. Flowering red sorrel ramet density and percent contribution of emerged red sorrel ramets to flowering ramet populations following Fall and Spring application of paraquat in two lowbush blueberry fields in Nova Scotia, Canada

Table 5.11. Mean pre and post-vernalization ramet counts and flowering frequency following pre and post vernalization clipping treatments applied to ramets established from planted root fragments

Table 5.12. Mean pre and post-vernalization seed plant leaf number, ramet density, and ramet leaf number for red sorrel plants established from seed and subject to pre and post-vernalization clipping treatments

Table 5.13. Weighted-Least-Squares ANOVA of flower frequency in red sorrel seed plants and ramets exposed to pre and post-vernalization clipping treatments
Table 5.14. Effect of pre and post-vernalization clipping of red sorrel ramets and seed plants on flower frequency, flower stem density, density of flowering ramets, and proportion of ramets flowering. ................................................................. 172

Table 5.15. Weighted-Least-Squares ANOVA for the saturated model for frequency of cell packs with flowering red sorrel ramets following exposure to constant or decreasing photoperiod and constant or decreasing temperature, with or without vernalization for 16 weeks at 6°C. ............ 176

Table 5.16. Weighted-Least-Squares ANOVA for the reduced model for frequency of cell packs with flowering red sorrel ramets following exposure to constant or decreasing photoperiod, with or without vernalization for 16 weeks at 6°C. ........................................................................... 176

Table 5.17. Frequency of cell packs with flowering red sorrel ramets, mean density, leaf number, stem height, and time to bolting of flowering ramets, and proportion of vernalized ramets flowering, in cell packs exposed to constant or decreasing photoperiod, with or without vernalization for 16 weeks at 6°C. ........................................................................... 177

Table 5.18. Final vegetative and flowering ramet density, ramet leaf number, and flowering ramet survival in cell packs exposed to constant or decreasing photoperiod with or without vernalization for 16 weeks at 6°C. ................................................................. 177

Table 6.1. GDD thresholds for proportional cumulative lowbush blueberry ramet emergence and development and corresponding GDD thresholds at alternative base temperatures determined by linear and quadratic regression of cumulative GDD ($T_{base}=0^\circ C$) against cumulative GDD calculated using the indicated base temperatures. ................................................................. 200

Table A.1. Application date, air temperature, wind speed, and relative humidity at time of MAT28 applications at Debert and Londonderry, Nova Scotia, in 2010. ............................................. 251

Table A.2. Herbicide treatments for goldenrod screening trial at Collingwood, Nova Scotia, in 2010. .................................................................................................................. 251

Table A.3. Application date, air temperature, wind speed, and relative humidity at time of PRE and POST herbicide applications at Collingwood, Nova Scotia, in 2010. ................. 252

Table A.4. Blueberry stem biomass and density in MAT28 dose response trial at Debert and Londonderry, Nova Scotia, in 2010. ........................................................................................................ 255

Table A.5. Blueberry stem heights and flower bud counts at Debert and Londonderry, Nova Scotia, in 2010. ........................................................................................................... 256

Table A.6. Goldenrod biomass and shoot density at 105 DAS in screening trial at Collingwood, Nova Scotia, in 2010. ........................................................................................................... 257
Table A.7. Goldenrod heights in PRE treatments at 42 and 105 DAS in MAT28 screening trial at Collingwood, Nova Scotia, in 2010. ................................................................. 257

Table A.8. Weed species, height, growth stage, and herbicide damage ratings in MAT28 spot spray trial in 2010. ................................................................. 258
List of Figures

Fig. 1.1. Recently established native stand of lowbush blueberry (Vaccinium angustifolium Ait.) in Nova Scotia, Canada................................................................. 2

Fig. 1.2. Established clones, or genets, of lowbush blueberry (Vaccinium angustifolium Ait.) in commercial field Nova Scotia, Canada................................................................. 2

Fig. 1.3. Rhizome and ramets of lowbush blueberry (Vaccinium angustifolium Ait.) .............. 3

Fig. 1.4. Pruning a lowbush blueberry (Vaccinium angustifolium Ait.) field with a flail mower after harvest in the bearing year................................................................. 4

Fig. 1.5. Emergence of new ramets of lowbush blueberry (Vaccinium angustifolium Ait.) from buds on the rhizomes (foreground) and base of cut stems (background). ......................... 5

Fig. 1.6. Emerged lowbush blueberry (Vaccinium angustifolium Ait.) ramet at A) tip dieback and B) with developing flower buds following transition to tip dieback. ......................... 6

Fig. 1.7. Lowbush blueberry (Vaccinium angustifolium Ait.) field in bloom during the spring of the bearing year.......................................................................................... 8

Fig. 1.8. Honey bee (Apis mellifera L.) on developing flower of red sorrel (Rumex acetosa L.) in a lowbush blueberry (Vaccinium angustifolium Ait.) field in Nova Scotia, Canada........... 9

Fig. 1.9. Infestation of red sorrel (Rumex acetosa L.) in a hexazinone-treated lowbush blueberry (Vaccinium angustifolium Ait.) field in Nova Scotia, Canada................................. 11

Fig. 1.10. Creeping root system and ramets of red sorrel (Rumex acetosa L.) in a lowbush blueberry (Vaccinium angustifolium Ait.) field in Nova Scotia, Canada................................. 12

Fig. 1.11. Lowbush blueberry (Vaccinium angustifolium Ait.), field with A) nearly complete clone coverage and B) less than 60% clone coverage. ................................................... 16

Fig. 1.12. Flowering stems of red sorrel (Rumex acetosa L.) covering lowbush blueberry (Vaccinium angustifolium Ait.) ramets................................................................. 19

Fig. 2.1. Daily mean air temperature (line) and rainfall (bars) during blueberry emergence and development to tip dieback at (A) Purdy-2009, (B) Wyvern-2009, (C) Pigeon Hill-2010, (D) Londonderry-2010, (E) Mount Thom-2011, and (F) North River-2011. ................................................... 32
Fig. 2.2. Mean daily air temperature (line) and rainfall (bars) during wild blueberry flowering at (A) Purdy-2010, (B) Wyvern-2010, (C) Pigeon Hill-2011, and (D) Londonderry-2011. .......... 33

Fig. 2.3. (A) Percent cumulative lowbush blueberry ramet emergence, (B) percent cumulative ramets at tip dieback, and (C) percent cumulative ramets with open flowers in relation to cumulative growing degree days (GDD) calculated from air temperature (T_{base} = 0^\circ C) at sites used for model calibration in Nova Scotia, Canada................................................................. 38

Fig. 2.4. Percent of total number of open blueberry flowers in relation to cumulative growing degree days (GDD) calculated from air temperature (T_{base} = 0^\circ C) at (A) Londonderry 2011 and (B) Pigeon Hill 2011......................................................................................................................... 42

Fig. 2.5. Observed and model predicted percent cumulative lowbush blueberry ramet emergence, (C-D) observed and model predicted percent cumulative lowbush blueberry ramets at tip dieback, and (E-F) observed and model predicted percent cumulative flowering lowbush blueberry ramets in relation to growing degree days (GDD) calculated from air temperature (T_{base} = 0^\circ C).......................................................................................................................... 44

Fig. 3.1. Cumulative new, dead, and net red sorrel ramets in blueberry patches at A) Purdy -2009, B) Purdy-2010, C) Wyvern-2009, D) Wyvern-2010, E) Pigeon Hill-2010, and F) Pigeon Hill-2011.......................................................................................................................... 72

Fig. 3.2. Cumulative new, dead, and net red sorrel ramets in bare soil patches at A) Purdy -2009, B) Purdy-2010, C) Wyvern-2009, D) Wyvern-2010, E) Pigeon Hill-2010, and F) Pigeon Hill-2011.......................................................................................................................... 73

Fig. 3.3. Cumulative new, dead, and net red sorrel seedlings at A) Purdy-2010, B) Wyvern-2010, C) Pigeon Hill-2010, and D) Pigeon Hill-2011. ........................................................................................................................................ 74

Fig. 3.4. Percent cumulative male flowering red sorrel ramets, female flowering red sorrel ramets, and female ramets with seeds at A) Purdy-2009, B) Purdy-2010, C) Wyvern-2009, D) Wyvern-2010, E) Pigeon Hill-2010, and F) Pigeon Hill-2011....................................................................................... 82

Fig. 3.5. Single-season depletion curves of flowering and vegetative overwintering red sorrel ramets in blueberry patches at A) Purdy-2010, B) Wyvern-2010, C) Pigeon Hill- 2010, and D) Pigeon Hill-2011....................................................................................... 83

Fig. 3.6. Single-season depletion curves of flowering and vegetative overwintering red sorrel ramets in bare soil patches at A) Purdy-2010, B) Wyvern-2010, C) Pigeon Hill- 2010, and D) Pigeon Hill-2011....................................................................................... 84
Fig. 3.7. Mean percent contribution of overwintering and monthly ramet cohorts to the final net ramet population in blueberry and bare soil patches at A) Purdy-2010, B) Wyvern-2010, C) Pigeon Hill-2010, and D) Pigeon Hill-2011...

Fig. 3.8. Life-cycle diagram describing the development and survival of red sorrel ramets and seedlings in non-bearing and bearing year lowbush blueberry fields in Nova Scotia, Canada...

Fig. 4.1. Daily mean air temperature (line) and rainfall (bars) during red sorrel ramet emergence and flowering at A) Purdy-2009, (B) Wyvern-2009, (C) Pigeon Hill-2010, (D) Purdy-2010, (E) Wyvern-2010, and (F) Pigeon Hill-2011...

Fig. 4.2. The relationship between the mean number of ramets per 2cm red sorrel root fragment and constant temperatures of 1, 2, 3, 4, and 5°C...

Fig. 4.3. The relationship between the mean number of ramets per 2cm red sorrel root fragment and constant temperatures of 5, 10, 15, 20, 25, and 35°C...

Fig. 4.4. Calibration of GDD (T_{base}=0°C) models for predicting A) emergence and B) flowering of red sorrel ramets in lowbush blueberry fields in Nova Scotia, Canada...

Fig. 4.5. Observed and model predicted red sorrel ramet emergence (A and B) and flowering (C and D) in relation to growing degree-days (GDD’s) calculated from air temperature (T_{base}=0°C)...

Fig. 5.1. Mean percent cumulative flowering red sorrel ramets in cell packs in the P16:VY treatment in the photoperiod X vernalization experiment after transfer to grow shelves following vernalization for 12 weeks...

Fig. 5.2. Quadratic relationship between red sorrel flower number per stem and height of the flowering stem...

Fig. 5.3. A) Number of cell packs with flowering seed plants or ramets after transfer to grow shelves following 5, 10, or 15 weeks of vernalization...

Fig. 5.4. Repeated measures ANOVA of mean net red sorrel ramet density in lowbush blueberry following Fall and Spring paraquat applications at A) Mt. Thom, and B) North River, Nova Scotia in 2010 and 2011...

Fig. 5.5. A) Number of pots with a flowering red sorrel seed plant or ramet and B) percent cumulative flowering ramets in the Clip-No treatment of the pre and post-vernalization ramet removal experiment following transfer to a 24.4 ± 0.2°C growth facility after vernalization at 4.5 ± 0.1°C for 12 weeks...
Fig. 5.6. Mean percent cumulative flowering of ramets in cell packs after transfer to the greenhouse following exposure to constant (n=14) or decreasing (n=32) photoperiod and vernalization for 16 weeks at 6°C. .................................................................................................................. 178

Fig. 6.1. Developmental stage of two lowbush blueberry (Vaccinium angustifolium Ait.) clones at the Debert Wild Blueberry Institute on May 16, 2013. .......................................................... 193

Fig. 6.2. Regression of cumulative growing degree-days calculated at a base temperature of 0°C on cumulative growing degree-days calculated at a base temperature of 6°C at the Purdy research site in Collingwood, Nova Scotia in 2010. .......................................................... 199

Fig. 6.3. Use of the proposed life-cycle model for Rumex acetosella L., modified from Chapter 3, to develop an herbicide rotation for resistance management in lowbush blueberry. ............... 209

Fig. 6.4. Overwintering ramet of red sorrel (Rumex acetosella L.) bolting in early May in a lowbush blueberry (Vaccinium angustifolium Ait.) field in Collingwood, Nova Scotia. .......... 215

Fig. A.1. Percent blueberry stem biomass reduction in preemergence MAT28 treatments in sprout year wild blueberry fields at A) Debert and B) Londonderry, Nova Scotia. ............... 254
List of Abbreviations

°C; degree Celsius
a.i.; active ingredient
ANOVA; analysis of variance
CL; confidence limit
cm; centimeter
\( \text{cm}^3 \); cubic centimeter
\( \text{CO}_2 \); carbon dioxide
DAT; days after transfer
DF; degrees of freedom
g; gram
GDD; growing degree-day
ha; hectare
\( \text{KaNo}_3 \); potassium nitrate
L; litre
m; meter
\( \text{m}^2 \); square meter
ml; millilitre
mm; millimeter
N; north
No.; number
P; p-value
PPFD; photosynthetic photon flux density
PSI; pounds per square inch
$R^2_{Adj}$; adjusted $R^2$

$Ro$; net ramet population growth rate

RMSE; root mean square error

SE; standard error

$T_{base}$; base temperature

$T_{mean}$; mean temperature

$W$; west

$X^2$; chi-square

XR; extended range
Chapter 1: General Introduction and Literature Review

1.1. Lowbush Blueberry Crop Value, Production, and Potential for Predictive Models

The lowbush blueberry (*Vaccinium angustifolium* Ait.) is an economically important fruit crop in Quebec and the Atlantic provinces of Canada and the state of Maine in the United States (Strik and Yarborough, 2005; Yarborough, 2004). Total acreage in these regions now exceeds 170,000 acres (Strik and Yarborough, 2005) with a combined production of nearly 172 million pounds of lowbush blueberries in 2010 (AAFC, 2012a; USDA, 2013). Nova Scotia is the second largest producer of lowbush blueberries in Atlantic Canada (AAFC, 2012b, 2012a). The production acreage in the province increased by 31% between 1992 and 2003 (Strik and Yarborough, 2005) and production is currently estimated to occur on just over 43,000 acres (AAFC, 2012a). Approximately 28 million pounds of lowbush blueberries were produced in Nova Scotia in 2011 with an estimated farm gate value of $22 million dollars (Statistics Canada, 2012).

The lowbush blueberry is a native, perennial berry species in Nova Scotia (Barker et al., 1964). Commercial fields are not planted but are developed on abandoned farmland or cleared woodland where native blueberry stands already exist (AAFC, 2012a; Barker et al., 1964) (Fig. 1.1). These stands are composed of distinct and variable clones that spread by underground rhizomes (Glass and Percival, 2000; Hall and Aalders, 1961; Nams, 1994). Each clone is a genetically unique individual, or genet, that has established naturally from seed at some point in the history of the stand (Bell et al., 2009a) (Fig. 1.2). Individual shoots, or ramets, that emerge from the rhizomes of each genet constitute the aboveground portion of each plant (Barker and
Collins, 1963) (Fig. 1.3), from which fruit is harvested. The majority of the blueberry acreage in Nova Scotia is managed on a two-year production cycle. Plants are pruned to ground level to

![Image](image1)

Fig. 1.1. Recently established native stand of lowbush blueberry (*Vaccinium angustifolium* Ait.) in Nova Scotia, Canada.

![Image](image2)

Fig. 1.2. Established clones, or genets, of lowbush blueberry (*Vaccinium angustifolium* Ait.) in commercial field Nova Scotia, Canada.
promote vigorous ramet growth from the rhizomes in the first, or non-bearing year (Barker et al., 1964; Black, 1963). Flowering, fruit development, and harvest occur in the second, or bearing year (Wood, 2004). A small portion of the acreage in Nova Scotia is also managed on a three-year cycle in which an additional bearing year is harvested prior to pruning (Eaton and Nams, 2006; Jordan and Eaton, 1995).

![Rhizome and ramets of lowbush blueberry](image)

**Fig. 1.3.** Rhizome and ramets of lowbush blueberry (*Vaccinium angustifolium* Ait.)

Pruning is accomplished with flail mowers or burning (Ismail and Hanson, 1982; Penney et al., 2008), either at the end of the last bearing year or early in the spring of the non-bearing year (Fig. 1.4); plants therefore regenerate naturally from rhizomes following pruning at the start of each production cycle (Hall et al., 1979). Most management practices in wild blueberry production are governed by the timing of ramet emergence or specific ramet growth stages. Blueberry growers rely on calendar dates (Aalders et al., 1972) or general references to crop development stage (Anonymous, 2012) for timing these management practices. The growth habit
of the lowbush blueberry, however, is similar to many perennial weeds whose emergence and development have been successfully modelled (Donald, 2000; Ekeleme et al., 2004; Satorre et al., 1985; Webster and Cardina, 1999). These models, often based on temperature or growing degree-days, lend predictive capabilities to the timing of weed management practices. Similar models for lowbush blueberry emergence and development would lend the same predictive capability to the timing of important crop and pest management practices during the blueberry production cycle.

Fig. 1.4. Pruning a lowbush blueberry (Vaccinium angustifolium Ait.) field with a flail mower after harvest in the bearing year.

1.2. Lowbush Blueberry Emergence and Development during the Two-Year Production Cycle

Growth and development of lowbush blueberry ramets in the non-bearing year is comprised of emergence and vegetative growth of ramets until abortion of the apical meristem (tip dieback) stimulates development of floral buds on the upper portion of ramets in late
summer and autumn (Aalders and Hall, 1964; Barker and Collins, 1963; Hall and Ludwig, 1961). Ramets emerge from the base of previously pruned stems and from buds on the rhizomes (Barker et al., 1964; Barker and Collins, 1963) (Fig. 1.5). Proportional emergence from each structure is dependent upon pruning method (Ismail and Hanson, 1982) with burning or flail mowing close to the soil surface favoring ramet emergence from rhizomes (Ismail and Yarborough, 1981; Kender et al., 1964). Most fields in Nova Scotia are pruned by flail mowing and therefore likely contain ramet populations originating from both structures.

Fig. 1.5. Emergence of new ramets of lowbush blueberry (*Vaccinium angustifolium* Ait.) from buds on the rhizomes (foreground) and base of cut stems (background).
Initiation of ramet emergence has traditionally been a function of temperature and date of spring pruning (Eaton and White, 1960; Trevett, 1962). Ramets emerged rapidly from mid to late May until late June following burn-pruning in late March in Maine (Eggert, 1957). Ramets also emerged in mid-May following spring burning in New Brunswick (Barker and Collins, 1963). Emergence ceases by mid-summer in commercial fields due to apical dominance of emerged ramets (Kender, 1968), though emergence of new ramets as late as early September was reported in spring-burned plots in Manitoba (Hoefs and Shay, 1981). Modern wild blueberry production systems in Nova Scotia typically employ autumn pruning after harvest in the bearing year instead of spring pruning in the non-bearing year. The effect of pruning date on ramet emergence is expected to be negligible in these systems, and initiation of ramet emergence in the non-bearing year is likely regulated by air or soil temperatures in early spring. Timing of ramet emergence is important for weed management as soil-applied herbicides such as hexazinone and terbacil need to be applied prior to blueberry emergence to prevent crop injury (Ismail et al., 1981; Jensen and Kimball, 1985; Jensen, 1985; Yarborough et al., 1986).

Fig. 1.6. Emerged lowbush blueberry (*Vaccinium angustifolium* Ait.) ramet at A) tip dieback and B) with developing flower buds following transition to tip dieback.
Emerged ramets exhibit a determinate growth habit (Bell, 1950) which culminates in abortion of the apical meristem, or tip dieback (Barker and Collins, 1963; Bell, 1953) (Fig. 1.6A). Tip dieback is thought to be inherently controlled (Barker and Collins, 1963) and contributes to the branched habit of unpruned plants (Jordan and Eaton, 1995). For example, Barker and Collins (1963) reported flush after flush of vegetative growth interrupted by tip dieback in softwood cuttings of *V. angustifolium* maintained under long days in the greenhouse. A similar growth habit is exhibited by several cultivars of highbush blueberry (*V. corymbosum*) as well (Gough et al., 1978). Tip dieback in lowbush blueberry generally begins in early summer (Barker and Collins, 1963; Percival et al., 2012) and is a prerequisite to flower bud initiation in the non-bearing year (Aalders and Hall, 1964; Bell, 1950; Bell and Burchill, 1955) (Fig. 1.6B). The period between ramet emergence and tip dieback is therefore crucial for establishment of both crop density and biomass that will support the development of flower buds for the bearing year. Weed control during this period increases ramet density and the number of flower buds per emerged ramet (Eaton, 1994; Jensen, 1985, 1986). Predicting the duration of this period could therefore provide a basis for understanding the potential competitive interactions between lowbush blueberry and associated weed species.

Growth and development in the bearing year is comprised of leaf expansion and flowering in spring (Bell and Burchill, 1955; Hall et al., 1979) and fruit development and harvest by late summer. Flower buds developed in the non-bearing year overwinter in a dormant state (Bell, 1953). Dormancy ends in March of the bearing year (Bell and Burchill, 1955) and bud swelling occurs in early May when air temperatures exceed 10°C for more than 4 days (Hall et al., 1979). Open flowers are generally observed by mid to late May with the duration of
flowering ranging anywhere from one to three weeks (Chiasson and Argall, 1996; Wood, 1961) (Fig. 1.7). Cross-pollination is essential for good fruit set (Aalders and Hall, 1961; Wood, 1968) and is routinely achieved through the use of native and introduced pollinators (Barker et al., 1964; Eaton and Nams, 2012; Yarborough, 2004). Weeds that flower during blueberry bloom can potentially interfere with pollination (Drummond et al., 2009) (Fig. 1.8) or, conversely, provide alternative forage options for native and introduced pollinators (Drummond et al., 2009; Hughes, 2012; Stubbs et al., 1992). Native bees that pollinate lowbush blueberry also benefit from the presence of plants that flower before and after the lowbush blueberry (Argall et al., 1998; MacKenzie et al., 2004, Stubbs et al., 1992), similar to that described for other crops (Nicholls and Altieri, 2012). A predictive model of blueberry flowering therefore provides a framework for understanding the ecological and management implications of the flowering time of weeds during the bearing year.

Fig. 1.7. Lowbush blueberry (Vaccinium angustifolium Ait.) field in bloom during the spring of the bearing year.
1.3. General Weed Situation, Status and Biology of *Rumex acetosella* in Lowbush Blueberry Fields in Nova Scotia

Weeds are one of the most limiting factors in the production of lowbush blueberries (Jensen, 1985; McCully et al., 1991). Regular pruning maintains the early succession environment inhabited by lowbush blueberry (Yarborough et al., 1986), but also promotes the persistence of many perennial weeds (Hall, 1959; Yarborough and Bhowmik, 1989; Yarborough and Ismail, 1985). Approximately 80 and 90% of the weed species identified in weed surveys in 1984-1985 and 2000-2001, respectively, were perennials (Jensen and Sampson, unpubl. Data; McCully et al., 1991). These weeds compete with the lowbush blueberry for resources such as space (Kinsman, 1993; Yarborough and Bhowmik, 1993), nutrients (Penney and McRae, 2000; Smagula and Ismail, 1981), and light (Hall, 1958); interfere with harvesting (Hanchar et al., 1985; Jensen and Specht, 2002; Kennedy et al., 2010; Yarborough et al., 1986), contribute
unwanted fruit to the harvested blueberry crop (McCully et al., 1991; Yarborough and Ismail, 1979, 1980), and reduce overall yields (Yarborough and Marra, 1997).

Red sorrel (*Rumex acetosella*) is the most common perennial species in commercially managed lowbush blueberry fields. Surveys of weedy vegetation in lowbush blueberry fields in Nova Scotia have documented a 43% increase in the occurrence of red sorrel between the early 1980’s and the early 2000’s (Jensen and Sampson, unpubl. Data; McCully et al., 1991). The plant is now established in over 90% of the lowbush blueberry acreage in Nova Scotia (Jensen and Sampson, unpubl. data). Seed of *R. acetosella* is a common contaminant on harvesting equipment (Boyd and White, 2009) and control from commonly used herbicides, such as hexazinone, is variable (Kennedy et al., 2010; Kennedy et al., 2011; Li, 2013) or unacceptable (Fig. 1.9). Control associated with autumn applications of pronamide is the most reliable option (Hughes, 2012), but is plagued by the unpredictability of climatic requirements essential for successful weed control with this product. Identification of new management strategies is hindered by limitations in the current level of knowledge of the population biology of *R. acetosella* in lowbush blueberry fields in Nova Scotia.
An extensive account of the biology of *Rumex acetosella* L. has recently been published (Stopps et al., 2011). Discussion of the biology of this species in the current document is therefore limited to information pertinent to the research reported herein. *R. acetosella* is an herbaceous creeping perennial (Sampson et al., 1990). The plant is dioecious and spreads by seeds and a shallow creeping root system (Kennedy, 2009; Kiltz, 1930). The creeping root system in established populations in lowbush blueberry fields in Nova Scotia is quite extensive (Fig. 1.10). Established populations in grass swards tend to be maintained predominantly by vegetative reproduction of ramets from the creeping root system (Putwain et al., 1968; Putwain and Harper, 1970). This has also been confirmed in established populations in lowbush blueberry fields in Nova Scotia (Kennedy, 2009) (Fig. 1.10). *Rumex acetosella* ramet populations are reported to peak in early to late spring and then decline throughout the season in lowbush blueberry fields (Kennedy et al., 2010). Similar patterns in *R. acetosella* ramet density have also
been reported in established ramet populations in grass swards (Putwain et al., 1968). These studies, however, provide little information regarding ramet dynamics in established populations or the factors contributing to the segregation of ramet development commonly observed in these populations.

Fig. 1.10. Creeping root system and ramets of red sorrel (*Rumex acetosella* L.) in a lowbush blueberry (*Vaccinium angustifolium* Ait.) field in Nova Scotia, Canada.

Established *R. acetosella* ramet populations are developmentally segregated and contain both vegetative and flowering ramets (Fujitaka and Sakai, 2007; Putwain and Harper, 1972). Flowering in field populations of *R. acetosella* ramets can occur throughout the season (Escarré and Thompson, 1991), but tends to be observed most frequently in early to mid-summer in most temperate regions (Fujitaka and Sakai, 2007; Harris, 1970; Korpelainen, 1992). *Rumex acetosella* ramets tend to flower in lowbush blueberry fields around mid-June in Nova Scotia (Kennedy, 2009). This generally follows the early-season peak in ramet density reported by Kennedy et al. (2010) and may indicate that flowering is confined to early-emerging ramets.
However, initial ramet counts at some study sites included what appeared to be overwintering ramets persisting from the previous season (Kennedy, 2009; Kennedy et al., 2010). *Rumex acetosella* can require more than one year of growth before initiating flowering under controlled conditions (Harris, 1970), but little is known about the factors affecting flowering of *R. acetosella* in Nova Scotia. The plant is reported to remain vegetative under short days (Listowski and Jackowska, 1964). Flowering will occur after 130 to 140 days under long days, but this duration is shortened to about 40 days when plants are grown under short days prior to transfer to long days (Bavrina et al., 1991). Flowering has also been reported to occur under long days and ambient greenhouse conditions (Lovett Doust and Lovett Doust, 1987; Zimmerman and Lechowicz, 1982). No studies, however, have been conducted to understand the factors regulating flowering of *R. acetosella* ramets under field conditions. It is therefore unclear as to why ramet populations of this species segregate to flowering and non-flowering ramets.

### 1.4. Improving the Current Knowledge of *Rumex acetosella* Ramet Dynamics with a Demographic Approach

No studies of *R. acetosella* have incorporated a demographic approach to understanding seasonal cycles of ramet birth and death in this species. As such, the true dynamics of *R. acetosella* ramet populations in lowbush blueberry fields have not been documented and are not well understood. Demography is the study of births, deaths, and the structure of populations (Harper and White, 1974). Important considerations when choosing census variables for demographic studies of plants, adapted from Maillette (1992) include 1) demographic competence of the census variables to be measured, 2) contribution of the census variables to the competitive ability and persistence of the studied species, 3) sensitivity of the census variables to experimental treatments or imposed conditions, and 4) considerations of practical limitations to
the collection of appropriate data. Plants can be considered as metapopulations of modules (White, 1979), and Harper (1980) was one of the first to suggest that modular units of plants, such as ramets, be considered as census variables and studied as such using a demographic approach. The demography of herbaceous creeping perennials such as *R. acetosella* can therefore be studied at the genet (i.e. genetic individual) or at the ramet (i.e. module of growth of the genetic individual) level of organization (Eriksson, 1989; Harper, 1980). The importance of genet-level studies of clonal plants has been stressed (Cook, 1983, 1985) and is especially important during the early phases of establishment (Hartnett and Bazzaz, 1985a). The identification of individual genets in field situations dominated by established herbaceous perennials is, however, extremely difficult and imposes a practical limitation on studies at the genet level. This practical limitation of genet identification in established *R. acetosella* populations in lowbush blueberry fields was recently confirmed (Kennedy et al., 2011). Changes in populations of established herbaceous perennials, however, can often be attributed primarily to changes occurring at the ramet level of organization (Bishop et al., 1978; Eriksson, 1993, 1988; Hartnett and Bazzaz, 1985a; Harper, 1980). *Rumex acetosella* ramets are readily identifiable in lowbush blueberry fields, and demography of ramets is an important first step towards understanding both the demography and clonal architecture of established genets (White, 1980; Wikberg and Svensson, 2003).

Ramel populations of herbaceous clonal perennials tend to be regulated by seasonal cycles of ramet birth and death (Cook, 1985; Harper, 1977) and thus meet an essential requirement for demographic study. Cook (1983, 1985) summarizes two important concepts with regards to the demography of ramet populations of clonal plants. First, ramet mortality tends to
be relatively constant over long periods of time (i.e. years) but is more variable within a given season. Second, net ramet density within a population remains relatively constant despite a great flux in ramet births and deaths over time. Both concepts can be seen in ramet populations originating from stolons in *Ranunculus* spp. (Lovett Doust, 1981; Sarukhan and Harper, 1973) and *Heiraceum pilosella* (Bishop et al., 1978) and rhizomes in *Carex arenaria* (Noble et al., 1979), *Rubia peregrina* (Navas and Garnier, 1990) and *Viola fimbrulata* (Solbrig et al., 1988). These regular cycles of ramet birth and death may be causally related (Harper, 1980; Noble et al., 1979) or simply result from ramet birth and death associated with routine growth of the genet (Cook, 1983, 1985). Regardless, ramet flux is important for the persistence of established genets at a given site. Studies of ramet demography in herbaceous creeping perennials that spread by creeping roots are lacking and none have been conducted for *R. acetosella*.

Finally, a demographic approach to studying plant populations can be useful for determining the effects of contrasting habitats imposed on ramet populations of established genets. Vegetative spread of established blueberry clones is very slow (Hall et al., 1979; Lapointe and Rochefort, 2001) with crop coverage ranging from near complete in mature fields (Hepler and Yarborough, 1991) to less than 60% in younger fields (Lapointe and Rochefort, 2001; Yarborough and Bhowmik, 1989) (Fig. 1.11). Field area not occupied by blueberry clones is occupied by bare ground or is colonized by weed species (Jensen and Yarborough, 2004; Lapointe and Rochefort, 2001). *Rumex acetosella* ramets grow both within blueberry clones, as well as the bare areas between blueberry clones “(White, personal observation)”. Ramet and seedling dynamics of some herbaceous perennial plants differ in contrasting habitats (Bishop et al., 1978; Bruun et al., 2007; Johnson and Thomas, 1978; Lesica and Ellis, 2010; LovettDoust,
1981), but this aspect of the ramet dynamics of *R. acetosella* have not been studied. A key component of lowbush blueberry management is to encourage the spread of blueberry clones and thus increase the habitat occupied by blueberry clones within commercially managed fields.

Fig. 1.11. Lowbush blueberry (*Vaccinium angustifolium* Ait). field with A) nearly complete clone coverage and B) less than 60% clone coverage.
(Hall et al., 1972; Kender and Eggert, 1966). This generally hinders the establishment and growth of weed species (Jensen and Yarborough, 2004; Yarborough and Bhowmik, 1993), though similar data for red sorrel are lacking.

### 1.5. Improving the Current Knowledge of Developmental Segregation in Established *Rumex acetosella* Ramet Populations with a Demographic Approach

Meristems of polycarpic herbaceous creeping perennials can develop vegetatively, sexually, or remain dormant (Bonser and Aarssen, 1996; Fagerström, 1992; Rohde and Bhalerao, 2007). An important aspect of the reproductive biology of these plants is the maintenance of a balance between vegetative and sexual meristems (Amasino, 2009; Battey, 2000), a factor critical to the success of many polycarpic herbaceous creeping perennial plants as weeds (Leaky, 1981). Vegetative meristems allow for perpetuation of successful genotypes in local populations, while sexual meristems allow for genetic recombination and dispersal to new sites via flowering and seed production (Abrahamson, 1980; Eriksson, 1993; Harper and White, 1974). A balance between vegetative and sexual meristems is maintained in herbaceous creeping perennials by production of dormant or non-dormant buds on underground perennial structures (Hartnett and Bazzaz 1985b; McAllister and Haderlie, 1985, Werner et al., 1980; White, 1979), or through the production of both vegetative and sexual (e.g. flowering) aboveground shoots, or ramets (Araki and Ohara, 2008; Nault and Gagnon, 1993; Noble et al., 1979; Slade and Hutchings, 1989; Worthen and Stiles, 1986).

Ramets of herbaceous creeping perennials can be monocarpic (Baskin and Baskin, 1988; Bishop et al., 1978; Noble and Marshall, 1983) or polycarpic (Araki and Ohara, 2008; Cain and Damman, 1997; Eriksson, 1988; Pitelka et al., 1985) with flowering thought to be controlled by
both biotic and abiotic factors (Cook, 1985). Biotic factors include the extent of physiological integration between interconnected ramets (Ashmun et al., 1982; Cain and Damman, 1997; Evans, 1992; Pitelka and Ashmun, 1985), ramet size (Baskin and Baskin, 1988; Eriksson, 1988; Grace and Wetzel, 1982; Méndez and Obeso, 1993; Pitelka et al., 1985; Schmid et al., 1995; Solbrig et al., 1988), competition from associated vegetation (Abrahamson, 1980; Hartnett and Bazzaz, 1985b), and ramet ontogeny and genetics (Charpentier and Stuefer, 1999; Pors and Werner, 1989). Abiotic factors affecting ramet flowering are primarily environmental variables such as temperature or vernalization (Heide, 1994, 1995; MacDonald et al., 1984; Walck et al., 1999) and photoperiod (Hunter and Smith, 1972; Kurepin et al., 2007; MacDonald et al., 1984).

The importance of abiotic factors in regulating ramet development in herbaceous perennials has been stressed (Cook, 1985; Sachs, 2002) and has been studied extensively in many ornamental (Cameron et al., 2007; Fausey et al., 2006), monocarpic (Klinkhamer et al., 1987b) and monocot herbaceous perennials (Greenup et al., 2009; Heide, 1994). Fewer studies have directly examined the role of these factors in ramet development of herbaceous creeping perennials that spread by creeping roots. The impact of biotic or abiotic factors on flowering of *R. acetosella* could be ascertained using a demographic approach to impose structure on established ramet field populations. Flowering and non-flowering *R. acetosella* ramets co-exist in these populations (discussed above) and flowering must therefore be regulated by some factor (i.e. ramet size or emergence timing) common to the majority of flowering ramets in any given year. Flowering stems of *R. acetosella* grow above blueberry stems and severely impede harvesting in bearing year fields (Fig. 1.12). Given the dioecious nature of the plant, synchronous flowering of male and female ramets is also essential for successful seed production that may or may not contribute to seasonal seedling recruitment in established populations.
1.6. Role of Seedling Recruitment in the Maintenance of Clonal Plant Populations

Established populations of clonal plants are subject to either initial or repeated seedling recruitment (Eriksson, 1989). Initial seedling recruitment is the result of seed germination due to a rare disturbance and generally results in low genetic diversity in established populations (Eriksson, 1989). In contrast, populations subject to repeated seedling recruitment acquire new genets from seed on a regular basis and therefore maintain genetically diverse populations (Coelho et al., 2008; Eriksson, 1989; Soane and Watkinson, 1979). Eriksson (1993) maintains that these two categories should be viewed as endpoints on a continuum of seedling recruitment in clonal plants as the density of established genets tends to reduce seedling establishment (Waite and Hutchings, 1979). Seedling recruitment in established populations of clonal plants therefore ranges from fairly frequent (Coelho et al., 2008; Newell et al., 1981) to rare (Lovett Doust, 1981; Navas and Garnier, 1990) to completely absent (Bishop et al., 1978; Dickerman
and Wetzel, 1985; Hartnett and Bazazz, 1985a; Pitelka et al., 1985). Establishment from seed is generally rare or completely absent in established populations of *R. acetosella* (Kennedy, 2009; Putwain et al., 1968; Putwain and Harper, 1970), despite rather high seed production in most populations (Escarré and Thompson, 1991; Kennedy, 2009). Seedling establishment was also reported to be less than 2% in an early successional environment in which the seedbank was dominated by *R. acetosella* (Marteinsdóttir et al., 2010). The role of seedling recruitment in the maintenance of established populations of *R. acetosella* in lowbush blueberry fields in Nova Scotia is still unclear. Seeds are readily dispersed by blueberry harvesters (Boyd and White, 2009), and the impacts of this dispersal on the genetic diversity of established populations may be underappreciated.

### 1.7. Research Objectives

The first objective of this research was to improve our ability to predict lowbush blueberry phenology in Nova Scotia through the assessment of growing degree-day models to predict ramet emergence and tip dieback in non-bearing fields and flowering in bearing fields. This research was based on the hypothesis that temperature, as measured by growing degree-days, accounts for most of the variation in emergence, tip-dieback, and flowering in lowbush blueberry. This research was conducted in several lowbush blueberry fields throughout North-Central Nova Scotia. It is anticipated that results of this objective will improve the timing of important management practices in lowbush blueberry production and provide a framework for relating crop development to that of weeds and other pests.

The second objective of this research was to conduct a demographic study of the herbaceous creeping perennial weed *R. acetosella* L. in lowbush blueberry fields in Nova Scotia. The study focused primarily on demography at the ramet level of organization, but attempts at
estimating annual seedling recruitment into established populations were also made. This research was based on the hypothesis that ramet dynamics of *R. acetosella* were regulated by seasonal cycles of birth and death, and that this cycle was unaffected by the presence or absence of blueberry clones. Demographic data was used to construct a life-cycle model of *R. acetosella* for the two-year lowbush blueberry production cycle. Data from this study were also used to determine the applicability of growing degree-day models for predicting ramet emergence and flowering of this species in Nova Scotia. This research was based on the hypothesis that temperature, as measured by growing degree-days, accounts for most of the variation in emergence and flowering of *R. acetosella* in lowbush blueberry fields in Nova Scotia. These models will then be used to relate ramet emergence and flowering of *R. acetosella* to that of the lowbush blueberry through results of objective one.

The third objective, which stemmed from the demographic study outlined above, was to investigate the role of abiotic factors on *R. acetosella* ramet sprouting and flowering. Specifically, this research was conducted to test the hypothesis that developmental segregation of *R. acetosella* ramets was regulated by a vernalization requirement for flowering. This research was conducted under controlled conditions using greenhouse and growth chamber facilities located at the Dalhousie Faculty of Agriculture campus located in Truro, Nova Scotia. It is anticipated that results of this objective will contribute greatly to improving our understanding of the role of abiotic factors in the regulation of ramet, and thus overall genet, development in *R. acetosella*. 
Chapter 2: Emergence and Development of Wild Blueberry (*Vaccinium angustifolium* Ait.) Ramets in Nova Scotia

This chapter is a modified version of the following manuscript published in HortScience:

2.1. Abstract.

Experiments were established to evaluate the suitability of growing degree day (GDD, \(T_{\text{base}}=0^\circ\text{C}\)) models for predicting emergence and development of wild blueberry ramets in Nova Scotia, Canada. Data for model development were collected from quadrats established in several non-bearing and bearing wild blueberry fields throughout the dominant wild blueberry production areas in Northern and Central Nova Scotia. Wild blueberry ramets emerged between 222 and 265 GDD (6 May to 14 May) and reached 90% emergence between 619 and 917 GDD. Emergence continued to slowly increase until late summer or early autumn. Tip dieback began between 598 and 792 GDD (14 June to 21 June) and duration of this phase depended on whether or not late emerging ramets developed to tip dieback. Open flowers were detected between 376 and 409 GDD (19 May to 30 May) in the bearing year. Duration of flowering was approximately 400 GDD. A four-parameter Weibull and a 3-parameter Gompertz equation adequately explained cumulative wild blueberry ramet emergence and cumulative ramets at tip dieback as functions of GDD in the non-bearing year, respectively. The four-parameter Weibull function also explained the relationship between cumulative ramets with open flowers and GDD in the bearing year. Model predictions for initiation of emergence, tip dieback, and flowering were 243, 692, and 389 GDD, respectively. Models were validated with independent data sets collected throughout Northern and Central Nova Scotia. The relationship between the percentage of open flowers and GDD in the bearing year was well described by a Gaussian model at two sites, with a predicted peak number of open flowers between 552 and 565 GDD.
2.2. Introduction

Commercial wild blueberry fields are developed from native stands (AAFC, 2005; Barker et al., 1964) composed of distinct and variable clones that spread by rhizomes (Glass and Percival, 2000; Hall and Aalders, 1961). The crop is managed on a two-year cycle. Plants are pruned to ground level to promote vegetative growth in the first, or non-bearing year. Flowering, fruit development, and harvest occur in the second, or bearing year. Early research in wild blueberries focused on factors affecting the growth and development of this species, but the trend since the late 1970’s has been towards applied research in pest and cultural management practices that improve yield. This research has been extremely successful in improving yields and profitability of wild blueberry production (Yarborough, 2004). However, few studies have focused on improving our understanding of factors affecting growth and development of wild blueberries in modern production systems.

Emergence and development timing of wild blueberries is primarily a function of environmental or inherent factors and generally cannot be adjusted by grower practices (e.g. planting date). Growth and development in the non-bearing year is comprised of emergence and vegetative growth of ramets until abortion of the apical meristem (tip dieback) stimulates development of floral buds on the upper portion of ramets in late summer and autumn (Aalders and Hall, 1964; Barker and Collins, 1963; Hall and Ludwig, 1961). Eggert (1957) reported that ramets emerged rapidly from mid to late May until late June, with initiation of emergence largely determined by temperature and date of spring pruning (Eaton and White, 1960; Trevett, 1959; Trevett, 1962). Trevett (1962) indicated that initiation of emergence was more rapid following
late spring pruning, primarily due to an increase in the rate of heat unit accumulation. Modern wild blueberry production systems in Nova Scotia typically employ autumn pruning after harvest in the bearing year instead of spring pruning in the non-bearing year. The effect of pruning date on ramet emergence is expected to be negligible in these systems, and initiation of ramet emergence in the non-bearing year is likely regulated by air or soil temperatures in early spring.

Vegetative growth of emerged ramets is most rapid in warm temperatures and long days (Hall and Ludwig, 1961; Kender, 1967). Tip dieback is thought to be inherently controlled and not induced by external stimuli (Barker and Collins, 1963). However, temperature is an important factor affecting apical shoot abortion in some woody species (Millington, 1963; Suzuki, 1991). Abortion of the apical meristem in wild blueberry is a prerequisite to flower bud initiation in the non-bearing year (Aalders and Hall, 1964; Bell, 1950; Bell and Burchill, 1955). Therefore, the time period between ramet emergence and tip dieback is crucial in terms of establishment of both crop density and biomass that will support the development of flower buds for the bearing year. Extensive stands of wild blueberries in certain regions of Nova Scotia regularly fail to produce acceptable fruit yields, primarily due to limitations imposed on growth and development by low temperatures in both the non-bearing and bearing years (Hall et al., 1964; Hall et al., 1970). Bell (1950) also reported considerable variation in the timing of blueberry development between locations from season to season, but this variability is reduced when comparing development at these locations within a single season. Therefore, rates of ramet emergence and development to tip dieback in the non-bearing year may simply be a function of temperature within a given growing season.
Ramet growth and development in the bearing year consists primarily of leaf expansion, flowering and fruit production (Bell and Burchill, 1955; Hall et al., 1979). Although flowering may be induced by short photoperiods (Hall and Ludwig, 1961), bud swelling occurs in early May when air temperatures exceed 10°C for more than 4 days (Hall et al., 1979). Timing of flowering is also delayed by cooler temperatures (Bell, 1953). The duration of flowering ranges anywhere from one to three weeks (Chiasson and Argall, 1996; Wood, 1961), and timing of pest control and pollinator movement is in direct response to the onset of flowering. Spring temperatures have been found to be a reliable predictor for budburst of vegetative buds in northern hardwood trees (Hunter and Lechowicz, 1992) and the evergreen *Picea sitchensis* (Cannell and Smith, 1983) under field conditions. NeSmith and Bridges (1992) were also able to use temperature to predict the onset of flowering of a Rabbiteye blueberry cultivar under controlled conditions. The use of temperature to predict the onset of wild blueberry flowering in the field would be useful to growers and field managers, particularly those responsible for ensuring the protection and pollination of large wild blueberry acreages.

Most management practices in wild blueberry production are governed by the timing of ramet emergence or specific ramet growth stages. Wild blueberry growers rely on calendar dates (e.g. Aalders et al., 1972) or general references to crop development stage (Anonymous, 2012) for timing these management practices. Published studies report the general timing of ramet emergence or growth stage in the field (Barker and Collins, 1963; Eggert, 1957; Wood, 1961), but the use of temperature or thermal time improves the ability to predict timing of plant emergence or specific plant growth stages (Martinson et al., 2007; O’Dell et al., 1999). Relating blueberry ramet emergence and growth stage to thermal time may also reduce the variability in
timing of these processes between field locations and growing seasons (Bell, 1950; Bell, 1953; Wood, 1961). Thermal models have been quite successful for predicting the emergence and development of crop species such as wheat (Cao and Moss, 1989; Davidson and Campbell, 1983), corn (Tollenaar et al., 1979; Warrington and Kanemasu, 1983), and canola (Vigil et al., 1997). Thermal models have also been useful for predicting harvest dates of highbush blueberry cultivars in Michigan (Carlson and Hancock Jr., 1991) and cumulative flowering of ‘Rabbiteye’ blueberries (NeSmith and Bridges, 1992). Similar models have not been developed for wild blueberries in Nova Scotia, but thermal models to predict maturity of perennial forage crops in this region have been successful (Bootsma, 1984). Although managed as a crop, the wild blueberry is a native species that is not planted. Therefore, plants regenerate naturally following pruning at the start of each production cycle. The wild blueberry is therefore similar to many perennial weeds whose growth and development have been successfully modelled using a temperature-based approach (Donald, 2000; Ekeleme et al., 2004; Satorre et al., 1985; Webster and Cardina, 1999). The effects of weather conditions have proven inconsistent in terms of predicting wild blueberry fruit production in Eastern Canada (Hall et al., 1982), but no attempts have been made to model growth and development as functions of growing degree days.

The wild blueberry acreage in Nova Scotia increased by 31% between 1992 and 2003 and is expected to exceed 40,000 acres by 2013 (Strik and Yarborough, 2005). This increased acreage, in combination with management practices driven by the growth and development of a native species in response to environmental conditions, give immediate applicability to predictive models in crop management. The objectives of this study were to 1) develop growing-degree day models to predict emergence and tip dieback of wild blueberry ramets in the non-
bearing year and flowering in the bearing year, and 2) validate these models with independent data sets collected throughout northern and central Nova Scotia.

2.3. Materials and Methods

2.3.1. Site Selection

Blueberry ramet emergence and development were monitored in six commercial wild blueberry fields in Nova Scotia between 2009 and 2011. Site locations were chosen based on proximity to large wild blueberry acreages in northern and central Nova Scotia. All sites were initially established in fields that had been pruned by flail mowing at the end of the previous season. Two sites were established in the non-bearing year near Collingwood in 2009 (Purdy and Wyvern), with an additional non-bearing year site near Collingwood in 2010 (Pigeon Hill) (Table 2.1). Non-bearing year sites were also established near Londonderry in 2010 and Mount Thom and North River in 2011 (Table 2.1). Sites established in non-bearing fields in 2009 and 2010 were retained for bearing year data collection in 2010 and 2011, respectively (Table 2.1).

2.3.2. Weather Data

Hourly air temperature at each site was monitored using temperature loggers (HOBO® Pro V2, Onset Computer Corporation, Cape Cod, Massachusetts). Data loggers were attached to wooden stakes and were located about 0.5m above the soil surface. Regional air temperature data from the nearest Environment Canada weather station were used to supplement field-based temperature data so that growing degree days (GDD) could be calculated starting on April 1 (day of year 91). GDD’s were calculated using the formula:

\[ GDD = \sum_{i=1}^{n} (T_{mean} - T_{base}) \]  

\[ [1] \]
where $T_{\text{mean}}$ is the mean daily air temperature, $T_{\text{base}}$ is the lowest air temperature at which it is assumed blueberry emergence or development will not occur, and $n$ is the number of days over which GDD’s are calculated. In this equation, $GDD = 0$ if $T_{\text{mean}} \leq T_{\text{base}}$, similar to the approach used by Gordon and Bootsma (1993) for determining annual GDD accumulations in Atlantic Canada. Rainfall data for each site were obtained from the nearest Environment Canada weather station. Mean daily air temperature and rainfall data for non-bearing and bearing year sites are provided in Figs. 2.1-2.2.
Table 2.1. Description of study sites used to collect data for calibration and validation of growing degree day models developed for lowbush blueberry in Nova Scotia, Canada. Data for development and validation of blueberry ramet emergence and tip dieback models were collected at non-bearing year sites. Data for development and validation of blueberry flowering models were collected at bearing year sites. Non-bearing year sites established in 2009 and 2010 were retained for bearing year data collection in 2010 and 2011, respectively.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Production Year</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>Soil Type$^z$</th>
<th>Soil pH$^y$</th>
<th>Soil %OM$^y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdy-2009</td>
<td>Non-bearing</td>
<td>45°35′34.904″ N</td>
<td>63°50′49.932″ W</td>
<td>114</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Purdy-2010</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyvern-2009</td>
<td>Non-bearing</td>
<td>45°32′57.042″ N</td>
<td>63°55′56.311″ W</td>
<td>238</td>
<td>Sandy loam</td>
<td>4.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Wyvern-2010</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon Hill-2010</td>
<td>Non-bearing</td>
<td>45°35′10.900″ N</td>
<td>63°51′37.525″ W</td>
<td>190</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Pigeon Hill-2011</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Londonderry-2010</td>
<td>Non-bearing</td>
<td>45°26′21.280″ N</td>
<td>63°32′44.640″ W</td>
<td>62</td>
<td>Sandy loam</td>
<td>4.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Londonderry-2011</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mount Thom-2011</td>
<td>Non-bearing</td>
<td>45°29′30.373″ N</td>
<td>62°59′25.200″ W</td>
<td>229</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>7.4</td>
</tr>
<tr>
<td>North River-2011</td>
<td>Non-bearing</td>
<td>45°27′54.431″ N</td>
<td>63°12′46.471″ W</td>
<td>92</td>
<td>Silt loam</td>
<td>-$^x$</td>
<td>-$^x$</td>
</tr>
</tbody>
</table>


$^y$pH and % OM (% organic matter) determined from 4 soil cores taken to a depth of 10 cm at each site. Cores were combined to form a composite sample for each site. Composite samples submitted to the Nova Scotia Department of Agriculture Provincial Analytical Laboratory for analysis.

$^x$Soil pH and %OM data for this site were unavailable.
2.3.3. Emergence and Development Data

Blueberry ramet emergence and development to the tip dieback stage was monitored in four 0.09m$^2$ quadrats established at non-bearing year sites in 2009, 2010, and 2011 (Table 2.1). Newly emerged blueberry ramets were counted and marked with colored elastic bands once or twice weekly from early May until late October. Dead ramets were counted to estimate ramet mortality, and elastics were removed. Ramets at tip dieback were counted and tagged with metal wire to prevent double counting. Ramets were considered to have reached tip dieback when the black tip associated with death of the apical meristem was observed (Barker and Collins, 1963). In the bearing year, blueberry ramets with at least one open flower were counted and marked with metal wire once or twice weekly starting in early spring and continuing until no new ramets with open flowers were detected. Flowers were considered open when internal structures of the blossom (e.g. pistils and stamens) were visible to the naked eye. Counts for emergence, ramets at tip dieback, and ramets with open flowers were used to determine percent cumulative emergence, ramets at tip dieback, and ramets with open flowers. Six additional 0.09m$^2$ quadrats were established at bearing year sites in 2011 to estimate the total number of open flowers per blueberry ramet during the bloom period. Ten blueberry ramets in each quadrat were tagged prior to the beginning of flowering, and the total number of open flowers on each tagged ramet were counted twice weekly until the end of the bloom period. Data for each of the 10 tagged ramets per quadrat were combined to obtain the mean percent of open flowers per quadrat on each counting date, and these data are presented as the mean percent of the total number of open flowers per ramet.
Fig. 2.1. Daily mean air temperature (line) and rainfall (bars) during blueberry emergence and development to tip dieback at (A) Purdy-2009, (B) Wyvern-2009, (C) Pigeon Hill-2010, (D) Londonderry-2010, (E) Mount Thom-2011, and (F) North River-2011. Mean daily air temperature was obtained from HOBO temperature loggers placed 0.5m above the soil surface at each site. Rainfall data for Purdy-2009, Wyvern-2009, and Pigeon Hill-2010 were obtained from the Environment Canada weather station located at Nappan, Nova Scotia (45°45’34.400” N, 64°14’29.200” W, elevation 19.80m). Rainfall data for Londonderry-2010, Mount Thom-2011, and North River-2011 were obtained from the Environment Canada weather station located at Debert, Nova Scotia (45°25’00.000” N, 63°28’00.000” W, elevation of 37.50m).
Fig. 2.2. Mean daily air temperature (line) and rainfall (bars) during wild blueberry flowering at (A) Purdy-2010, (B) Wyvern-2010, (C) Pigeon Hill-2011, and (D) Londonderry-2011. Mean daily air temperature was obtained from HOBO temperature loggers placed 0.5m above the soil surface at each site. Rainfall data for Purdy-2010, Wyvern-2010, and Pigeon Hill-2011 were obtained from the Environment Canada weather station located at Nappan, Nova Scotia (45°45’34.400” N, 64°14’29.200” W, elevation 19.80m). Rainfall data for Londonderry-2011 were obtained from the Environment Canada weather station located at Debert, Nova Scotia (45°25’00.000” N, 63°28’00.000” W, elevation of 37.50m).
2.3.4. Development of Thermal Models

Cumulative blueberry ramet emergence, ramets at tip dieback, and ramets with open flowers were plotted as functions of GDD. Fitting of non-linear equations, as well as parameter estimates for these equations, was conducted using the Gauss-Newton algorithm in PROC NLIN of the SAS system for Windows Version 9.2 (SAS Institute, Cary, NC). Cumulative blueberry ramet emergence \( Y \) was related to cumulative GDD with a Weibull equation of the form:

\[
Y = k\left[1 - \exp\left(-b(GDD - m)^c\right)\right]
\]

where \( Y \) is cumulative percent emergence at any given GDD, \( k \) is the theoretical maximum cumulative emergence, \( b \) is the rate of increase in emergence, \( m \) is the lag phase until the onset of emergence, and \( c \) is a shape parameter (Ekeleme et al., 2004; Martinson et al., 2007). The base air temperature for blueberry ramet emergence was determined by iterating a series of base temperatures (0 to 5°C in 1°C intervals) in equation 2 until the best fit was obtained between cumulative ramet emergence and cumulative thermal time (Izquierdo et al., 2009). The best fit was obtained for \( T_{\text{base}} \) equal to 0°C. Although a \( T_{\text{base}} \) of 1.7°C has previously been recommended for lowbush blueberry emergence (Trevett, 1959), 0°C was chosen based on best fit and simplicity in data calculation in both the current study and for potential end-users of the proposed model.
Cumulative blueberry ramets at tip dieback (Y) was related to cumulative GDD with a Gompertz equation of the form:

\[ Y = k \times \exp[\exp(-b(GDD - m))] \]  \[3\]

where \( Y \) is cumulative ramets at tip dieback, \( k \) is the theoretical maximum cumulative ramets at tip dieback, \( b \) is the rate of increase in the percentage of ramets at tip dieback, and \( m \) is the inflection point of the curve on the x-axis (Dorado et al., 2008). Cumulative ramets with open flowers in the bearing year was related to GDD using the Weibull equation described above, where \( Y \) is cumulative percent ramets with open flowers, \( k \) is the theoretical maximum cumulative ramets with open flowers, \( b \) is the rate of increase in cumulative ramets with open flowers, \( m \) is the lag phase until the onset of flowering, and \( c \) is a shape parameter.

The mean percent of total open flowers was related to GDD with a Gaussian equation of the form:

\[ Y = k \left[ \exp \left( -0.5 \left( \frac{GDD - m}{b} \right)^2 \right) \right] \]  \[4\]

where \( Y \) is the mean percent of total open flowers, \( k \) is the theoretical maximum percent of total open flowers, \( m \) is the GDD at the maximum percent of total open flowers, and \( b \) is a shape parameter. Using the iterative procedure described above, \( T_{\text{base}} \) values between 0 and 5°C provided similar fit of the proposed tip tieback and flowering models (based on criteria described below). A \( T_{\text{base}} \) value of 0°C was, therefore, maintained for GDD calculations to allow for use of
the same GDD scale when comparing thermal requirements for emergence, tip dieback, and flowering.

2.3.5. Assessing Fit and Validation of Thermal Models

Goodness of fit for all models was determined by calculating the coefficient of determination ($R^2$) and adjusted coefficient of determination ($R^2_{Adj}$):

$$R^2 = 1 - \frac{\sum (y_{obs} - y_{pred})^2}{\sum (y_{obs})^2}$$  \hspace{1cm} [5]

and

$$R^2_{Adj} = 1 - \frac{n(1-R^2)}{n-p}$$  \hspace{1cm} [6]

where $y_{obs}$ and $y_{pred}$ are the observed and predicted values, respectively, $n$ is the number of observations and $p$ is the number of parameters in the regression equation (Bowley, 2008), and the root-mean-square-error (RMSE):

$$RMSE = \sqrt{\frac{1}{n} \sum (y_{obs} - y_{pred})^2}$$  \hspace{1cm} [7]

Goodness of model fit was based on low RMSE and $R^2_{Adj}$ values close to 1, and these formulae and criteria were used when assessing fit of all models proposed in this study.
Blueberry ramet emergence and tip dieback development models were validated with emergence and tip dieback data from two non-bearing year sites that were not used for model calibration (Wyvern 2009 and North River 2011) (Table 2.1). The model for predicting cumulative percent ramets with open flowers was validated at two sites in the Collingwood area (Wyvern 2010 and Pigeon Hill 2011) (Table 2.1). Emergence and development data were expressed as percent cumulative emergence and development and plotted against air GDD. Emergence and development predictions were calculated with the models and plotted against actual emergence and development, and the $R^2_{\text{Adj}}$ and RMSE described above were used to assess agreement between observed data and model predictions. The Gaussian model fit to the percent of total open flowers was not validated due to lack of additional site-years of data.

2.4. Results

2.4.1. General Trends in Emergence, Tip Dieback, and Flowering

Wild blueberry ramets emerged between 222 and 265 GDD (6 May to 14 May) at all study sites (Fig. 2.3A). Emergence up to 90% was rapid, but this phase was followed by a much slower emergence period and ramet populations peaked between 2132 and 2768 GDD (13 Sept. to 20 Oct.) (Fig. 2.3A). Survival of emerged ramets in the non-bearing year was greater than 90% at all sites. Ramets were first observed at tip dieback between 598 and 792 GDD (14 June to 21 June) (Fig. 2.3B), which coincided with 72 to 96% blueberry ramet emergence. Duration of tip dieback ranged from 42 to 119 days and the percent of ramets at tip dieback peaked between 1529 and 2480 GDD (29 July to 11 Oct.) (Fig. 2.3B). Blueberry flowering in the bearing year began between 376 and 409 GDD (19 May to 30 May) and continued for 21 to 42 days after initiation (Fig. 2.3C). The
Fig. 2.3. (A) Percent cumulative lowbush blueberry ramet emergence, (B) percent cumulative ramets at tip dieback, and (C) percent cumulative ramets with open flowers in relation to cumulative growing degree days (GDD) calculated from air temperature ($T_{\text{base}} = 0^\circ\text{C}$) at sites used for model calibration in Nova Scotia, Canada. Symbols represent the mean of 4 observations. Lines are fitted regression equations. A Weibull equation of the form $Y = k[1 - \exp(-b(GDD - m))]$ was fit to percent cumulative ramet emergence and percent cumulative flowering ramets. A Gompertz equation of the form $Y = k \times \exp[-\exp(-b(GDD - m))]$ was fit to percent cumulative ramets at tip dieback. Parameter estimates and goodness of fit statistics for each regression equation are given in Table 2.2.
percentage of ramets with open flowers peaked between 692 and 748 GDD (16 June to 17 June) (Fig. 2.3C), but the maximum percentage of total open flowers was recorded between 538 and 564 GDD (7 June) (Fig. 2.4). Less than 5% of the total number of open flowers remained by 748-773 GDD (23 June) (Fig. 2.4).

2.4.2. Development of Thermal Models

Plotting ramet emergence, ramets at tip dieback, and ramets with open flowers as functions of GDD generally reduced the variability between sites observed when data were plotted as a function of day of year. The proposed models provided good fit to the field data and accurately predicted cumulative emergence, ramets at tip dieback, and ramets with open flowers as functions of cumulative GDD (Fig. 2.3; Table 2.2). Model predictions for the initiation of emergence and tip dieback in the non-bearing year were 243 and 692 GDD, respectively, while 389 GDD were predicted to accumulate before the initiation of flowering in the bearing year. Model predictions for 10, 50, 90, and 95% emergence, ramets at tip dieback, and ramets with open flowers are provided in Table 2.3. Both the Weibull and Gaussian models gave similar predictions for the initiation of flowering, and the Gaussian model predicted the maximum percentage of total open flowers by 552 and 565 GDD at Pigeon Hill and Londonderry, respectively, in 2011 (Fig. 2.4; Table 2.4). According to the Gaussian model, the bloom period lasted for just over 400 GDD at the study sites used to develop the model in 2011 (Fig. 2.4).

2.4.3. Model Validation

Model predictions for wild blueberry ramet emergence, ramets at tip dieback, and ramets with open flowers agreed quite closely with the observed values (Fig. 2.5), indicating good performance of these models for predicting emergence and development of wild
Table 2.2. Parameter estimates and goodness of fit statistics for proposed Weibull and Gompertz equations describing the relationship between growing degree days (GDD) calculated from air temperature ($T_{\text{base}} = 0^\circ\text{C}$) and percent cumulative lowbush blueberry ramet emergence, percent cumulative ramets at tip dieback, and percent cumulative flowering ramets in Nova Scotia, Canada. The Weibull equation was of the form $Y = k \left[1 - \exp\left(-b(GDD - m)\right)\right]$ and the Gompertz equation was of the form $Y = k \times \exp\left[-\exp\left(-b(GDD - m)\right)\right]$.

<table>
<thead>
<tr>
<th>Model</th>
<th>Site-Year</th>
<th>Equation</th>
<th>$k$</th>
<th>$b$</th>
<th>$m$</th>
<th>$c$</th>
<th>$R^2_{\text{Adj}}$</th>
<th>RMSE $^\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Cumulative Ramet</td>
<td>Purdy-2009</td>
<td>Weibull</td>
<td>96.9818</td>
<td>0.00179</td>
<td>242.5</td>
<td>1.1766</td>
<td>0.99</td>
<td>5.91</td>
</tr>
<tr>
<td>Emergence</td>
<td>Pigeon Hill-2010</td>
<td></td>
<td></td>
<td>(0.8376)$^x$</td>
<td>(0.00154)</td>
<td>(16.2539)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Londonderry-2010</td>
<td></td>
<td></td>
<td>(0.3508)</td>
<td>(0.00154)</td>
<td>(16.2539)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mount Thom-2011</td>
<td></td>
<td></td>
<td>(0.3508)</td>
<td>(0.00154)</td>
<td>(16.2539)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent Cumulative Ramets</td>
<td>Purdy-2009</td>
<td>Gompertz</td>
<td>99.7882</td>
<td>0.00486</td>
<td>1005.4</td>
<td>---</td>
<td>0.99</td>
<td>6.61</td>
</tr>
<tr>
<td>at Tip Dieback</td>
<td>Pigeon Hill-2010</td>
<td></td>
<td></td>
<td>(1.4101)</td>
<td>(0.000343)</td>
<td>(10.5541)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Londonderry-2010</td>
<td></td>
<td></td>
<td>(1.4101)</td>
<td>(0.000343)</td>
<td>(10.5541)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mount Thom-2011</td>
<td></td>
<td></td>
<td>(1.4101)</td>
<td>(0.000343)</td>
<td>(10.5541)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent Cumulative Flowering Ramets</td>
<td>Purdy-2010</td>
<td>Weibull</td>
<td>100.2</td>
<td>0.000950</td>
<td>383.7</td>
<td>1.4533</td>
<td>0.99</td>
<td>2.33</td>
</tr>
<tr>
<td></td>
<td>Londonderry-2011</td>
<td></td>
<td></td>
<td>(1.3937)</td>
<td>(0.00154)</td>
<td>(8.1976)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^x$Weibull model parameters, $k =$ theoretical maximum percent cumulative ramet emergence or percent cumulative flowering ramets, $b =$ rate of increase in percent cumulative ramet emergence or percent cumulative flowering ramets, $m =$ the lag phase until the onset of emergence or flowering, and $c =$ shape parameter (Martinson et al., 2007); Gompertz model parameters, $k =$ theoretical maximum percent cumulative ramets at tip dieback, $b =$ rate of increase in percent cumulative ramets at tip dieback, and $m =$ inflection point of the curve on the x-axis (Dorado et al., 2008).

$^\gamma$RMSE = Root mean square error.

$^x$Parentheses indicate SE of parameter estimates.
Table 2.3. Estimated growing degree days (GDD) calculated from air temperature ($T_{\text{base}} = 0^\circ$C) to reach 10, 50, 90, and 95 percent cumulative lowbush blueberry ramet emergence, percent cumulative ramets at tip dieback, and percent cumulative flowering ramets in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>10</th>
<th>50</th>
<th>90</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Cumulative Ramet Emergence</td>
<td>Weibull</td>
<td>276</td>
<td>407</td>
<td>734</td>
<td>928</td>
</tr>
<tr>
<td>Percent Cumulative Ramets at Tip Dieback</td>
<td>Gompertz</td>
<td>834</td>
<td>1082</td>
<td>1473</td>
<td>1626</td>
</tr>
<tr>
<td>Percent Cumulative Flowering Ramets</td>
<td>Weibull</td>
<td>410</td>
<td>477</td>
<td>595</td>
<td>636</td>
</tr>
</tbody>
</table>

$^a$GDD estimates from calibrated Weibull and Gompertz equations described in Table 2.2.
Table 2.4. Parameter estimates and goodness of fit statistics for the Gaussian model describing the relationship between growing degree days (GDD) calculated from air temperature \(T_{\text{base}} = 0^\circ\text{C}\) and percent of the total number of open flowers on lowbush blueberry ramets in Nova Scotia, Canada. The Gaussian model was of the form

\[
Y = k \left[ \exp \left( -0.5 \left( \frac{GDD - m}{b} \right)^2 \right) \right].
\]

<table>
<thead>
<tr>
<th>Site-year</th>
<th>Model Parameters(^z)</th>
<th>(R^2_{\text{Adj}})</th>
<th>RMSE(^y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Londonderry-2011</td>
<td>(k = 78.9965) (b = 69.9381) (m = 564.5)</td>
<td>0.99</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>(1.5143)(^x) (1.6720) (1.6552)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon Hill-2011</td>
<td>(k = 73.5606) (b = 75.1617) (m = 551.6)</td>
<td>0.97</td>
<td>5.24</td>
</tr>
<tr>
<td></td>
<td>(4.0392) (5.3868) (5.3029)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^z\)Gaussian model parameters, \(k = \) theoretical maximum percent of total open flowers, \(b = \) shape parameter, and \(m = \) GDD at the maximum percent of total open flowers (e.g. peak bloom).

\(^y\)RMSE = Root mean square error.

\(^x\)Parentheses indicate SE of parameter estimates.

Fig. 2.4. Percent of total number of open blueberry flowers in relation to cumulative growing degree days (GDD) calculated from air temperature \(T_{\text{base}} = 0^\circ\text{C}\) at (A) Londonderry 2011 and (B) Pigeon Hill 2011. Symbols are the mean of 6 observations. Lines are a fitted Gaussian equation of the form

\[
Y = k \left[ \exp \left( -0.5 \left( \frac{GDD - m}{b} \right)^2 \right) \right].
\]

Parameter estimates and goodness of fit statistics for the Gaussian equation fit to data for each site are given in Table 2.5.
blueberry ramets in Nova Scotia. All models had high $R^2_{Adj}$ and low RMSE (Table 2.5), indicating that temperature is likely the dominant factor affecting emergence and development of wild blueberry ramets in Nova Scotia. The proposed emergence model predicted a slightly faster rate of emergence at North River than was observed at this site, but the onset of ramet emergence was accurately predicted at both validation sites (Fig. 2.5A-B). The proposed tip dieback model predicted a slightly faster rate of cumulative ramets at tip dieback at North River and slightly earlier onset of this developmental phase at Wyvern, but predicted values were in close agreement with observed values at both sites (Fig. 2.5C-D; Table 2.5). Model predictions from the Weibull function used to predict flowering were in close agreement with observed flowering at both Wyvern and Pigeon Hill (Fig. 2.5E-F; Table 2.5).

Table 2.5. Goodness of fit statistics for validation of proposed Weibull and Gompertz models for predicting the relationship between growing degree days (GDD) calculated from air temperature ($T_{base} = 0^\circ C$) and percent cumulative lowbush blueberry ramet emergence, percent cumulative ramets at tip dieback, and percent cumulative flowering ramets in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Model Validated</th>
<th>Site-Year</th>
<th>Equation</th>
<th>$R^2_{Adj}$</th>
<th>RMSE$^z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Cumulative Ramet</td>
<td>North River-2011</td>
<td>Weibull</td>
<td>0.99</td>
<td>4.12</td>
</tr>
<tr>
<td>Emergence</td>
<td>Wyvern-2009</td>
<td></td>
<td>0.99</td>
<td>1.68</td>
</tr>
<tr>
<td>Percent Cumulative Ramets at Tip</td>
<td>North River-2011</td>
<td>Gompertz</td>
<td>0.99</td>
<td>5.33</td>
</tr>
<tr>
<td>Dieback</td>
<td>Wyvern-2009</td>
<td></td>
<td>0.99</td>
<td>5.57</td>
</tr>
<tr>
<td>Percent Cumulative Flowering</td>
<td>Wyvern-2010</td>
<td>Weibull</td>
<td>0.99</td>
<td>7.34</td>
</tr>
<tr>
<td>Ramets</td>
<td>Pigeon Hill-2011</td>
<td></td>
<td>0.99</td>
<td>3.71</td>
</tr>
</tbody>
</table>

$^z$RMSE = Root mean square error
Fig. 2.5. Observed and model predicted percent cumulative lowbush blueberry ramet emergence, (C-D) observed and model predicted percent cumulative lowbush blueberry ramets at tip dieback, and (E-F) observed and model predicted percent cumulative flowering lowbush blueberry ramets in relation to growing degree days (GDD) calculated from air temperature ($T_{\text{base}} = 0^\circ\text{C}$). Symbols are the mean of 4 observations, except at North River-2011 where symbols are the mean of 3 observations. Lines are calibrated model predictions. The calibrated model for predicting percent cumulative ramet emergence and percent cumulative flowering ramets was a Weibull equation of the form $Y = k[-\exp(-b(GDD - m))]$. The calibrated model for predicting percent cumulative ramets at tip dieback was a Gompertz equation of the form $Y = k\times\exp[-\exp(-b(GDD - m))]$. Goodness of fit statistics for assessing agreement between observed and model predicted values are given in Table 2.5.
2.5. Discussion

The pattern of wild blueberry ramet emergence observed in our study was similar to that reported by Hoefs and Shay (1981) following burning of a small clearing within a Pinus banksiana stand in Manitoba. Although timing of emergence initiation tended to be later than that observed in the current study, Hoefs and Shay (1981) reported rapid initial emergence in early June followed by a slower period of emergence that continued until late August or early September. In contrast, initiation of ramet emergence following burn-pruning in early spring in both New Brunswick (Barker and Collins, 1963) and Maine (Eggert, 1957; Trevett, 1959) was similar to that reported in the current study. The duration of emergence, however, was much shorter in Maine than that observed in Nova Scotia. Duration of blueberry ramet emergence in Maine is thought to be regulated by apical dominance preventing the emergence of new ramets throughout the latter portion of the non-bearing year (Kender, 1968; Trevett, 1962). The onset of the slow emergence period observed in our study coincided with the initial detection of dead ramets. Hall et al. (1969) reported increases in lateral bud activity following the release of apical dominance in wild blueberry, so the late season emergence observed in our study was likely due to the release of apical dominance by dying ramets. Matlack et al. (1993) also reported that mortality of dominant meristems was required for shoot regeneration in black huckleberry (Gaylussacia baccata), a rhizomatous shrub very similar in growth form to wild blueberries. It should also be noted that Kender (1968) identified a period of rhizome bud inactivity in late spring and early summer that was not induced by apical dominance. Kender (1968) referred to this as an inherent rest period in rhizome buds that ended around mid-summer. The start of the slower emergence period in the current study generally coincided with the end of the rest period.
reported by Kender (1968). No reports of this period of bud inactivity could be found for wild blueberry in Nova Scotia. Therefore, the extent of this phenomenon and its interactions with apical dominance in wild blueberry clones in this region are unclear. Nonetheless, data from the current study suggest that blueberry ramets rapidly approach peak emergence until the onset of ramet mortality, after which ramet emergence occurs slowly in response to the low mortality rate of already established ramets. Further study on the decline in rhizome bud activity in wild blueberry clones in Nova Scotia would be necessary before firm conclusions could be made regarding the effect of this phenomenon on the ramet emergence pattern observed in this study.

Previous reports describing the onset and duration of tip dieback vary in consistency with that observed in the current study. Eggert (1957) reported both a later initiation of tip dieback and a much shorter duration of this developmental phase in burn-pruned blueberry clones in Maine. Burning tends to remove all above-ground vegetation and stimulates ramet emergence from buds on underground rhizomes, whereas mowing only partially removes the above-ground vegetation and can stimulate emergence from buds on both rhizomes and the base of cut stems (Ismail and Hanson, 1982; Kender et al., 1964). Burning has therefore been found to promote a more uniform stand of unbranched ramets (Ismail et al., 1981). This might explain differences in the onset and duration of tip dieback in mowed blueberry fields in Nova Scotia when compared to burned fields in Maine. However, timing and duration of tip dieback in blueberry ramets emerging from burned plots in Manitoba and New Brunswick was similar to that reported in the current study (Hoefs and Shay, 1981; Barker and Collins, 1963). Also, the duration of tip dieback development in the current study seemed to be affected by the tendency of later emerging ramets to transition to this developmental stage. In some years (e.g. Purdy 2009) many
later emerging ramets did not progress to this stage, while in other years (e.g. North River 2011) later emerging ramets followed a developmental pattern similar to earlier emerging ramets. Trevett (1962) reported that later emerging ramets develop to the tip dieback stage, though often at a reduced height when compared to earlier emerging ramets. This was observed in the current study, as no ramets emerging after 90% emergence attained similar levels of growth as ramets emerging prior to this stage. The requirement for a critical level of vegetative growth prior to tip dieback development is generally unaccepted (Barker and Collins, 1963). Differences in growth of later emerging ramets therefore likely doesn’t explain why some developed to tip dieback and others did not. Poor growth and development of wild blueberry ramets has been reported under shaded conditions (Hall, 1958; Hall and Ludwig, 1961), so shading by earlier emerging ramets may have affected growth and development of later emerging ramets. Finally, Trevett (1962) has also suggested that tip dieback development can be inhibited by low leaf nitrogen levels inhibiting the synthesis of auxins required to induce abortion of the apical meristem. Leaf nitrogen levels in ramets emerging in early spring tend to decline until mid-summer before slowly increasing again (Townsend and Hall, 1970). It might be possible that leaf nitrogen levels in late emerging ramets could not be replenished in time for transition to tip dieback development at some of the sites in this study. In the end, the contribution of ramets emerging late in the non-bearing year to final yield in the bearing year is unclear, and some emphasis should be placed on determining the survival and contribution of these ramets to flowering and fruit production in the bearing year.

Timing of flowering was similar to that expected for a typical year in Nova Scotia (Chiasson and Argall, 1996; Wood, 1961), though the duration of flowering was somewhat
longer than might be expected based on other studies. Bell (1950) reported full blueberry bloom by the third or fourth week of May, about 7-10 days earlier than was observed in our study.

This study was the first attempt to develop growing-degree day models to predict wild blueberry emergence and development in Nova Scotia. Though previous attempts have been made to predict blueberry ramet emergence using growing degree days in Maine (Trevett 1959), the creation of these models is a major step forward in terms of improving our ability to predict the timing of emergence and development of this species in Nova Scotia. Timing of emergence, onset of tip dieback, and flowering were in general agreement with field observations in Atlantic Canada (Barker and Collins, 1963; Eaton and White, 1960; Wood, 1961). This suggests that the data sets used in this study for calibration and validation of the models were a good representation of these processes in modern wild blueberry production systems in Nova Scotia. General patterns in our data were also comparable to those of wild blueberry in other regions of Canada (Hoefs and Shay, 1981), which may make these models applicable in other blueberry growing regions of Canada.

Wild blueberry ramet emergence as a function of thermal time was very similar across sites (Fig. 2.3A), and validation of the proposed model indicated good agreement between model predictions and field observations (Fig. 2.5A-B). Based on degrees Fareinheit, Trevett (1959) estimated ramet emergence to occur after an average accumulation of 349 growing degree days following spring burning in Maine. Based on a conversion factor obtained from Miller et al. (2001), this is approximately equal to 194 growing degree days on a degrees Celsius scale. Ramet emergence therefore appears to occur more rapidly as a function of thermal time in Maine.
than in Nova Scotia. The primary herbicide used for weed control in wild blueberries in Nova Scotia (hexazinone) is applied pre-emergence to blueberries to prevent unacceptable crop injury (Jensen, 1985), giving the proposed emergence model for Nova Scotia utility in predicting the application timing of hexazinone and other pre-emergence herbicides. Fertilizer applications are generally made in early spring of the non-bearing year as well, so application of herbicides and fertilizers prior to 243 GDD should prevent unacceptable crop injury and ensure adequate nutrient status of the crop. Timing of ramet emergence is also important for monitoring some insect pests as well (Yarborough, 2001).

Moisture was not limiting during the emergence period at any of the study sites (Figs. 1-2). This may have facilitated the relatively uniform emergence across study sites. However, the effects of limited soil moisture on vegetative growth of the wild blueberry are generally minimal (Benoit et al., 1984; Glass et al., 2005). Moisture conditions in this study were also typical for early spring in Nova Scotia, so the results from this study are applicable to a typical growing season. Hydrothermal models, incorporating both temperature and moisture conditions into the predictive process, have been successful with some perennial species (Ekeleme et al., 2004). However, the results of this study suggest that a thermal-based model is sufficient for modelling wild blueberry ramet emergence.

Accumulation of growing degree days was a good predictor of tip dieback as model predictions from the proposed Gompertz model agreed quite closely with the field observations used to validate the model (Fig. 2.5C-D). While other factors can affect apical shoot abortion in blueberry (Hall and Ludwig, 1961; Kender, 1967) and other woody species (Barros and Neill,
our data indicate that temperature alone is adequate for predicting tip
dieback in blueberry under field conditions in Nova Scotia. Fertility recommendations for wild
blueberries are based on nutrient levels in leaf samples collected at 90-100% tip dieback in the
non-bearing year (Smagula and Yarborough, 2010), so leaf samples should be collected around
1600 GDD in non-bearing fields in Nova Scotia (95% tip dieback prediction, Table 2.3). In terms
of pest management, changes in the traditional weed flora of wild blueberry fields are resulting
in the requirement for more post-emergent herbicide options for annual and perennial weed
management (Jensen and Yarborough, 2004). Tolerance of wild blueberry to herbicides in the
non-bearing year, however, tends to vary with the developmental stage of the wild blueberry at
the time of herbicide application (Boyd, unpublished data). Combining predictions of ramet
emergence with predictions of tip dieback development in the non-bearing year will be useful in
future evaluations of new herbicides for managing increasingly diverse weed populations (Jensen
and Yarborough, 2004).

While short photoperiods have been associated with flowering in wild blueberries (Hall
and Ludwig, 1961), temperature is also an important factor affecting opening of wild blueberry
flower buds in spring (Hall et al., 1979; Wood, 1961). Our results indicate that temperature is the
main factor driving the rate of wild blueberry flowering in the field in Nova Scotia. Predictions
from our proposed Weibull model were in close agreement with field observations (Fig. 2.5E-F)
and can be used to facilitate disease monitoring at early bloom and improve timing of bearing-
year hexazinone applications (Delbridge et al., 2011; Jensen, 2002). When combined with the
results from the Gaussian model (Fig. 2.4; Table 2.4), both the onset and duration of flowering
can be predicted and used to improve pollinator management. When comparing the results of the
Weibull flowering model to those of the Gaussian model, it can be seen that flowering begins around 389 GDD and the number of open flowers peaks less than 200 GDD later. Individual wild blueberry flowers persist for 7 to 10 days and are most receptive to pollination within the first 5 days after opening (Wood, 1962). Introduced pollinators should be moved into wild blueberry fields by 390 GDD to ensure the presence of pollinators at the beginning of flowering. Pollinators should be removed around 570 GDD as the peak number of flowers has been reached by that time and pollinators may be more efficiently used at other locations. At the very least, the results of this study show that growers should remove pollinators by 400 GDD after the onset of flowering as the majority of blueberry blossoms have likely dropped by this time and alternative foraging options for these pollinators may be limited.

2.6. Conclusions

The results from this study provide strong evidence that temperature is one of the most important factors regulating the emergence and development of wild blueberry ramets in Nova Scotia. The growing-degree day models presented can be used to accurately predict blueberry emergence, tip dieback, and flowering in Nova Scotia. These models have utility in the timing of pest, fertility, and pollinator management during the 2-year wild blueberry production cycle. Additional emergence and development data sets should be collected throughout regions of Nova Scotia outside the range of this study to provide further validation or improvement of the models. Wild blueberry acreages in other provinces in Atlantic Canada have increased substantially over the past 20 years (Strik and Yarborough, 2005), so attempts to validate the proposed models in these regions, as well as other important production regions such as Maine, will be important for assessing the applicability of the models outside of Nova Scotia. Ripening of wild blueberry fruit is also affected by temperature (Hall and Aalders, 1968; Gibson, 2011), and development of
thermal models to predict ripening and harvest dates for wild blueberries would be a practical extension of this study.

3.1. Abstract

The dynamics and phenological development of *Rumex acetosella* L. ramets and seedlings were monitored within and between blueberry clones over the two-year lowbush blueberry production cycle in three commercial blueberry fields in Nova Scotia, Canada. A distinct overwintering red sorrel ramet population was identified and constituted the majority (>70%) of the population of flowering ramets. The actual proportion of the overwintering population that flowered, however, ranged from 39-75%. New ramets emerging in May and June also contributed to the population of flowering ramets, but no ramets emerging between July and November flowered in the year of emergence. Emergence of new ramets was season-long in both the non-bearing and bearing year, and ramet populations were regulated by a cycle of ramet birth and death in each year. Ramet populations within blueberry patches had higher growth rates and lower mortality than ramet populations in bare soil patches in the non-bearing year. As a result, large net gains to ramet populations occurred in blueberry patches in the non-bearing year. Ramet population growth rates and mortality were similar across blueberry and bare soil patches in the bearing year, and populations of net ramets tended to decline or stabilize during this year of the production cycle. The majority of the *R. acetosella* root system (>80%) was located in the top 0-7cm soil depth at all study sites and this likely contributed to similar emergence patterns across sites. Survival rates of overwintering and new ramet cohorts was variable, but ramets from both the initial overwintering population and monthly cohorts of new ramets contributed to a distinct age structure of net ramet populations at the end of each season. Seedling emergence was generally season-long in each year of the production cycle. Seedling survival ranged from 6 ± 6
to 51 ± 12% across sites but did not vary between blueberry and bare soil patches. No seedlings flowered in the year of emergence. Transition probabilities of ramets and seedlings derived from field data were used to develop a life-cycle model of red sorrel for the 2-year lowbush blueberry production cycle. This model should be useful for developing and assessing the impact of new management strategies for red sorrel in lowbush blueberry.
3.2. Introduction

The demography of herbaceous creeping perennials can be studied at the genet or ramet level of organization (Eriksson, 1989; Harper, 1980). Genet-level studies are important (Cook, 1983, 1985), particularly during the early stages of plant establishment (Hartnett and Bazzaz, 1985a). However, the identification of individual genets in field situations dominated by established herbaceous creeping perennials is often extremely difficult or impossible. Furthermore, changes in populations of established herbaceous creeping perennials can often be attributed primarily to changes occurring at the ramet level of organization (Bishop et al. 1978; Eriksson, 1993; Harper, 1980). Demography of ramets is an important first step towards understanding the demography of genets (White, 1980) and can improve our understanding and management of herbaceous creeping perennials that persist as weeds in certain situations.

Occurrence of red sorrel (R. acetosella), a common herbaceous creeping perennial species in lowbush blueberry fields in Nova Scotia, increased by 43% between the early 1980’s and the early 2000’s (Jensen and Sampson, unpubl. data). The plant is now established in over 90% of the lowbush blueberry acreage in Nova Scotia (Jensen and Sampson, unpubl. data). Red sorrel is dioecious and spreads by seeds and a shallow creeping root system (Kennedy, 2009). The role of seedling establishment in population maintenance is variable and considered minor in most established field populations (Putwain and Harper, 1970). Rather, these populations tend to be maintained predominantly by vegetative reproduction of ramets from the creeping root system (Putwain et al., 1968; Putwain and Harper, 1970). Ramet populations of this species tend to occur in distinct patches of varying size in lowbush blueberry fields, and attempts at whole plant
harvesting indicate the presence of multiple genets inhabiting these patches (Kennedy et al., 2011). Seedling survival within these patches is low (Kennedy, 2009) and the population seems to be regulated primarily at the ramet level.

Ramet populations of herbaceous creeping perennials tend to be regulated by seasonal cycles of ramet birth and death (Cook, 1985; Harper, 1977). Ramet mortality tends to be relatively constant over long periods of time (i.e. years) but is subject to more variation within a given season (Cook, 1985). Ramet density within a population therefore tends to remain relatively constant over time despite a great flux in actual ramet numbers (Cook, 1985). Both concepts can be seen in ramet populations originating from stolons in *Ranunculus* spp. (Lovett Doust, 1981; Sarukhan and Harper, 1973) and *Heiraceum pilosella* (Bishop et al., 1978). However, very little work has been done on ramet populations initiated from buds on creeping roots (Cook, 1985). Red sorrel ramet populations are reported to peak in early to late spring and then decline throughout the season in lowbush blueberry fields (Kennedy et al., 2010). Similar patterns in red sorrel ramet density have also been reported in grass swards (Putwain et al., 1968). However, these studies have not incorporated a demographic approach to understanding seasonal cycles of ramet birth and death in this species. As such, the true dynamics of red sorrel ramet populations in lowbush blueberry fields have not been documented and are not well understood.

Understanding the demography of a plant population allows for identification of specific individuals of interest (Booth et al., 2003). Ramet populations of many clonal plants are composed of flowering and non-flowering ramets (Araki and Ohara, 2008; Noble et al., 1979;
Worthen and Stiles, 1986) and this has been observed in field populations of red sorrel ramets as well (Fujitaka and Sakai, 2007; Putwain and Harper, 1972). Flowering in field populations of red sorrel ramets can occur throughout the season (Escarré and Thompson, 1991), but tends to be observed most frequently in early to mid-summer in most temperate regions (Fujitaka and Sakai, 2007; Harris, 1970; Korpelainen, 1992). Red sorrel ramets tend to flower in lowbush blueberry fields around mid-June in Nova Scotia (Kennedy, 2009). This generally follows the early-season peak in ramet density reported by Kennedy et al. (2010) and may indicate that flowering is confined to early-emerging ramets. However, initial ramet counts at some study sites included what appeared to be overwintering ramets persisting from the previous season (Kennedy, 2009; Kennedy et al., 2010). Red sorrel can require more than one year of growth before initiating flowering under controlled conditions (Harris, 1970), possibly indicating that overwintering ramets play an important role in the sexual reproduction of red sorrel in lowbush blueberry fields. Similar results have also been reported in field populations of ramets in other clonal species (Noble et al., 1979) but have not been investigated in field populations of red sorrel.

Finally, a demographic approach to studying plant populations can be useful for determining the effects of contrasting habitats on population dynamics of plant species. The lowbush blueberry is a native perennial berry species in Nova Scotia. Commercial fields are comprised of numerous blueberry clones that spread across the landscape through an extensive rhizome system. Due to the clonal nature of the lowbush blueberry, many fields contain a mixture of intermingled blueberry clones separated by bare soil areas that have yet to be colonized. Red sorrel ramets grow both within blueberry clones, as well as the bare areas between blueberry clones. Ramet dynamics of some herbaceous perennial plants differ in
contrasting habitats (Bishop et al., 1978; Lesica and Ellis, 2010; Lovett-Doust, 1981), but this aspect of the ramet dynamics of red sorrel have not been studied. A key component of lowbush blueberry management is to encourage the spread of blueberry clones and thus increase the habitat occupied by blueberry clones within commercially managed fields. This generally hinders the establishment and growth of weed species (Jensen and Yarborough 2004; Yarborough and Bhowmik, 1993), though similar data for red sorrel are lacking.

The objectives of this study were to 1) determine seasonal emergence, mortality, and net gain to red sorrel ramet populations in blueberry and bare soil patches in lowbush blueberry fields, 2) determine which ramet cohorts contribute to the flowering population of red sorrel ramets in lowbush blueberry fields, and 3) develop a preliminary estimate of seedling recruitment to established red sorrel populations in lowbush blueberry.

3.3. Materials and Methods

3.3.1. Description of Study Sites and Quadrat Placement

Four lowbush blueberry fields in Nova Scotia consisting of patchy distribution of blueberry clones with interspersed populations of red sorrel ramets were selected for this study. Two study sites were established in the spring of the non-bearing year in 2009 prior to blueberry emergence, with two additional sites established in Autumn 2009 following the pruning operation (Table 3.1). A total of eight quadrats were established for monitoring emergence and development of red sorrel ramets at each site; four in red sorrel patches occurring within blueberry clones and four in red sorrel patches occurring in bare soil areas between blueberry clones (hereby refered to as quadrat cover). Quadrat size was 0.09 m$^2$, and all quadrat locations
Table 3.1. Description of study sites used for collection of red sorrel ramet and seedling demographic data in lowbush blueberry fields in Nova Scotia, Canada. Non-bearing year sites established in 2009 and 2010 were retained for bearing year data collection in 2010 and 2011, respectively.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Production Year</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>Soil Type</th>
<th>Soil pH</th>
<th>Soil %OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdy-2009</td>
<td>Non-bearing</td>
<td>45°35’34.904” N</td>
<td>63°50’49.932” W</td>
<td>114</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Purdy-2010</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyvern-2009</td>
<td>Non-bearing</td>
<td>45°32’57.042” N</td>
<td>63°55’56.311” W</td>
<td>238</td>
<td>Sandy loam</td>
<td>4.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Wyvern-2010</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon Hill-2010</td>
<td>Non-bearing</td>
<td>45°35’10.900” N</td>
<td>63°51’37.525” W</td>
<td>190</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Pigeon Hill-2011</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Londonderry-2010</td>
<td>Non-bearing</td>
<td>45°26’21.280” N</td>
<td>63°32’44.640” W</td>
<td>62</td>
<td>Sandy loam</td>
<td>4.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Londonderry-2011</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


\(^{y}\)pH and % OM (% organic matter) determined from 4 soil cores taken to a depth of 10 cm at each site. Cores were combined to form a composite sample for each site. Composite samples submitted to the Nova Scotia Department of Agriculture Provincial Analytical Laboratory for analysis.
established in the non-bearing year were retained for bearing-year data collection at each site. Four additional 0.09m$^2$ quadrats were established in both blueberry clones and bare soil patches at all sites in 2010 to estimate seedling recruitment. Quadrats established for monitoring seedlings at Pigeon Hill in 2010 were retained for monitoring seedlings at this site in 2011.

Data collection at the Londonderry site (Table 3.1) was hindered by accidental overlap of an herbicide application by the blueberry grower that damaged initial ramet populations. However, limited data collection was still conducted at this site over the 2-year cycle to gain additional data for comparison purposes.

3.3.2. Red Sorrel Ramet and Seedling Dynamics

Newly emerged red sorrel ramets and seedlings were counted and carefully marked with colored elastic bands once or twice weekly from early May until early December. A different elastic color was used on each counting date to keep emergence cohorts separate. Counts were conducted more frequently during periods of rapid emergence or phenological development, and less frequently when little change in emergence or development was observed. Dead ramets and seedlings were counted and elastics were removed. Elastic color of dead ramets was not recorded in 2009 but was recorded at all sites in 2010 and 2011.

In early spring of the bearing year all surviving ramets from the non-bearing year in each quadrat were counted and old elastics were replaced with new elastics of consistent color. This ensured that all new ramets emerging in the bearing year were kept separate from old ramets persisting from the non-bearing year. The same approach was used for keeping older seedlings separate from new seedlings in seedling quadrats established in the non-bearing year at Pigeon
Hill in 2010. Only bearing-year seedling data were available for the Purdy and Wyvern sites. New and dead ramet and seedling counts from each site were used to determine cumulative new, dead, and net gains to ramet and seedling populations at each site.

For each production year at each site the initial ramet density, the density of new ramets produced, overall ramet mortality, final net ramet density, and the net ramet population growth rate for ramet populations in both blueberry and bare soil patches were calculated. Initial ramet density for the non-bearing year was simply the number of ramets counted and marked at the time of quadrat establishment; initial ramet density in the bearing year was the total number of surviving non-bearing year ramets remarked with consistent elastic colors in early spring of the bearing year. Density of new ramets in each quadrat was calculated as the total number of ramets counted in each season, minus the initial ramet density. Ramet mortality was expressed as the percentage of the total number of counted ramets that died. Net ramet population growth rate ($R_o$) was calculated using the following formula (Silvertown, 1982):

$$R_o = \frac{N_{t+1}}{N_t}$$

where $N_{t+1}$ is the final net ramet population for a given year and $N_t$ is the initial ramet population in that given year. Equation 1 was also used to determine $R_o$ values for ramet populations over the entire 2-year production cycle (production cycle $R_o$), where $N_{t+1}$ was the final bearing year net ramet density and $N_t$ was the initial ramet density at the time of quadrat establishment in the non-bearing year. For seedlings, the total density of new seedlings emerged, percent survival of
emerged seedlings, and final net seedling density for blueberry and bare soil patches were calculated at each site.

3.3.3. Red Sorrel Root Depth

The distribution of red sorrel roots with soil depth was determined in an effort to estimate the recruitment depth of ramets from the creeping root system. Red sorrel root depth was estimated from root biomass collected from cylindrical soil cores taken to depths of 7, 14, and 21 cm in three lowbush blueberry fields in 2012 (Table 3.1). Cores were collected using a soil bulk density core sampler with a core volume of 331 cm³. A total of 12 cores at each depth were collected from each site; six cores at each depth from red sorrel patches occurring within blueberry clones and six cores at each depth from red sorrel patches occurring in bare soil patches between blueberry clones. Cores were collected from all three sampling depths at each of the 12 core sampling locations. Cores were placed in paper bags in the field and stored in a cooler until returning to the lab. Cores were sieved in a 5.5 mm soil sieve to separate roots from the surrounding soil. Roots were gently rinsed in standing water, placed in paper bags, and dried for 48 hours at 70°C prior to weighing.

3.3.4. Ramet and Seedling Phenological Development

Phenological development of red sorrel ramets was monitored and related to time of ramet emergence based on the color of the elastic previously used to mark each ramet. Ramets were classified as either vegetative or flowering as no other obvious stage-classes were apparent in the field populations studied. All flowering ramets at each count were examined with a hand lens to identify male and female flower organs (stamens and pistils), and the total number of identifiable male and female flowering plants were counted and marked with colored paper clips.
to keep flowering ramets separated over counting dates. Similarly, all female ramets initiating seed set were counted and marked with colored paper clips at each site in 2010 and 2011, but not in 2009. The contribution of each ramet cohort to the total population of flowering ramets was assessed by determining the proportion of ramets from each elastic color that comprised the total population of flowering ramets. All new ramets contributing to the flowering population were grouped by month of emergence to simplify data presentation, and the data are presented as percent contribution of the initial and monthly ramet cohorts to the total population of flowering ramets.

3.3.5. Ramet Cohort Survival, Age Structure, and Life-Cycle Model

Cohort-specific ramet survival and net ramet population age structure were determined at all sites in 2010 and 2011. Ramets were grouped into the initial and subsequent monthly cohorts, and percent ramet survival for each cohort was determined based on the elastic colors of dead ramets recorded at each site. Survival data for each cohort were then used to determine the percent contribution of the initial and monthly ramet cohorts to final net ramet populations at each site in 2010 and 2011. Transition probabilities for the percentage of overwintering and new ramets flowering or remaining vegetative, survival of new and overwintering flowering and vegetative ramets, winter survival of final net ramet populations, and seedling survival were obtained directly from the field data and used to construct a life-cycle model of red sorrel for the 2-year lowbush blueberry production cycle. Since elastic colors of dead ramets were not recorded in the non-bearing year at Purdy-2009 and Wyvern-2009, survival probabilities for overwintering and new ramets in the non-bearing year were based on data from Pigeon Hill-2010 only.
3.4. Data Analysis

3.4.1. Red Sorrel Ramet and Seedling Dynamics

All new, dead, and net ramet and seedling data were analyzed separately at each site due to differences in both the total number of counts, as well as the temporal spacing between counts at each site. A repeated measures ANOVA on the initial non-bearing year, final non-bearing year, initial bearing year, and final bearing year net ramet density was used to determine if ramet data could be combined across quadrat cover at each site. The analysis was conducted with PROC MIXED in SAS (Version 9.2, Raleigh, NC) where counting date (day of year) was the repeated effect and quadrat cover, day of year, and the subsequent interactions were modeled as fixed effects. A spatial power covariance structure that assumed homogeneous variance over time (Bowley, 2008) was employed due to the unequal temporal spacing that occurred between the four counting dates. Net ramet density at Pigeon Hill and Wyvern were LOG(x) transformed and data at the Purdy site were squareroot(x) transformed to help the datasets meet the requirements for the assumptions of the ANOVA.

Differences in initial ramet density, the density of new ramets produced, overall ramet mortality, final net ramet density, and the net ramet population growth rate, between ramet populations in blueberry and bare soil patches at each site were determined by t-tests (PROC GLM, SAS Version 9.2, Raleigh, NC). Assumptions of normality and constant variance were confirmed for all means comparison by inspection of residual plots and normality tests in PROC GLM. The total density of new seedlings emerged, percent seedling survival, and final net seedling population density were compared across both types of quadrat cover at each site by t-tests (PROC GLM, SAS Version 9.2, Raleigh, NC).
The effects of site, quadrat cover, and the site X quadrat cover interaction on the production cycle $Ro$ values were determined using ANOVA in PROC GLM (SAS Version 9.2, Raleigh, NC). Production cycle $Ro$ values were $\log(x)$ transformed prior to the ANOVA to meet the assumptions of the analysis. The difference between production cycle $Ro$ values between blueberry and bare soil patches at each site was determined by a t-test (PROC GLM, SAS Version 9.2, Raleigh, NC). Data for Purdy and Pigeon Hill were $\log(x)$ transformed to meet the assumptions for the variance analysis, and back transformed means are presented for interpretation. All effects were considered significant at the 0.05 significance level.

3.4.2. Root Biomass

The effects of site, core depth, core location (blueberry or bare soil), and the subsequent interactions on root biomass were determined using ANOVA (PROC MIXED, SAS Version 9.2, Raleigh, NC). Root biomass values for the combined data set were $x^{0.25}$ transformed prior to analysis. Differences in root biomass at each core depth and core location were determined by ANOVA (PROC MIXED, SAS Version 9.2, Raleigh, NC). Means were generated using the LSMEANS statement, and differences were considered significant based on a Tukey’s test at a significance level of 0.05. Root biomass values in blueberry and bare soil patches were square root ($x$) and $\log(x)$ transformed, respectively, to meet the assumptions of the variance analysis. Means ± SE are presented on the transformed scale, and back-transformed means are presented for interpretation.

3.4.3. Ramet Cohort Survival, Age Structure, and Life-Cycle Model

The effect of site, quadrat cover, and the site X quadrat cover interaction on percent survival of final non-bearing year net ramet populations into the bearing year was determined by
ANOVA using PROC GLM (SAS Version 9.2, Raleigh, NC). ANOVA (PROC GLM, SAS Version 9.2, Raleigh, NC) was used to determine if there was a significant interactive effect between ramet cohort and quadrat cover on the percent contribution of the overwintering and monthly ramet cohorts to the final net ramet population age structure at each site. Percent contributions for each cohort at Purdy-2010 and Wyvern-2010 were squareroot(x) transformed prior to analysis. Percent contributions for each cohort at Pigeon Hill-2011 were LOG(x) transformed prior to analysis. The interaction was considered significant at the 0.05 level and was used to determine if the percent contribution of each cohort to final net ramet populations could be combined across both types of quadrat cover at each site.

Transition probabilities for new and overwintering ramets in non-bearing and bearing year fields were analyzed separately. The effect of site and quadrat cover on the probability of new and overwintering ramets flowering or remaining vegetative was determined using ANOVA (PROC GLM, SAS Version 9.2, Raleigh, NC). Differences in transition probabilities across sites were assessed by a Tukey’s means comparison and differences in transition probabilities between both types of quadrat cover within and across sites were determined by t-tests in PROC GLM (SAS Version 9.2, Raleigh, NC). Data transformations (LOG(x) and squareroot(x)) were employed as required to meet the assumptions of the variance analyses.

3.4.4. Ramet and Seedling Phenology

The effects of cohort, quadrat cover, and the cohort X quadrat cover interaction on the percent contribution to the total number of flowering ramets was determined by ANOVA (PROC GLM, SAS Version 9.2, Raleigh, NC). Monthly cohorts that did not contribute to the flowering population were not included in the analysis. All effects were considered significant at the 0.05
level, and mean percent contributions for each cohort were separated by a Tukey’s means comparison. In cases where only two cohorts contributed to the flowering population, significant differences were determined using a t-test (PROC GLM, SAS Version 9.2, Raleigh, NC). The segregation of the overwintering ramet population into flowering and vegetative ramets in blueberry and bare soil patches was tested by chi-square analysis of a 50:50 segregation using PROC FREQ (SAS Version 9.2, Raleigh, NC). The sex ratio of ramet populations were determined by summing the total number of identified male and female flowering ramets in both blueberry and bare soil patches in each production year at each site. The number of identified male and female ramets were combined across blueberry and bare soil patches at each site due to inconsistencies in the presence of both male and female ramets in each quadrat. The segregation of male and female ramets in the summed data was tested by chi-square analysis of a 50:50 segregation using PROC FREQ (SAS Version 9.2, Raleigh, NC). Deviation from a 50:50 segregation of male and female ramets was based on Yates corrected chi-square statistic in all cases where the total number of male and female ramets was less than 200 (Bowley, 2008).

The total number of flowering male and female ramets, as well as female ramets setting seed, were expressed on a percent cumulative scale and simply plotted as functions of day of year. Quadrats in some years contained only male or female flowering ramets. As a result, percent cumulative flowering data for male and female ramets were combined across both types of quadrat cover at each site to improve mean estimates of flowering at each site.
3.5. Results

3.5.1. Red Sorrel Ramet and Seedling Dynamics

There was a significant day of year (p ≤ 0.0002) and quadrat cover X day of year interaction effect (p ≤ 0.0267) on net ramet density at each site. Therefore, cumulative new, dead, and net ramet density are presented separately for both types of quadrat cover at each site (Figs. 3.1 and 3.2). Initial non-bearing year ramet density ranged from 222 ± 33 to 725 ± 68 (mean ± SE) ramets m⁻² but did not vary significantly by quadrat cover at each site (Table 3.2). Emergence of new ramets in the non-bearing year was season-long (Figs. 3.1 and 3.2) and the density of new ramets produced was generally higher in blueberry than in bare soil patches (Table 3.2). Non-bearing year ramet mortality was significantly lower in blueberry than in bare soil patches (Table 3.2). As a result, final net ramet density in the non-bearing year was significantly higher in blueberry patches (Table 3.2). Non-bearing year Ro values for ramet populations in blueberry patches ranged from 4.0 ± 0.5 to 5.4 ± 0.9 (mean ± SE) and were significantly greater than Ro values for ramet populations in bare soil patches at Wyvern and Pigeon Hill but not Purdy (Table 3.2).

Ramet survival into the bearing year did not vary significantly by site (p=0.1656) or quadrat cover (p=0.6156) and averaged 88 ± 3% (mean ± SE). Emergence of new ramets in the bearing year was season-long (Figs. 3.1 and 3.2) and the density of new ramets produced was once again generally higher in blueberry than in bare soil patches (Table 3.2). Ramet mortality, however, was high in both blueberry and bare soil patches and did not vary significantly across either type of quadrat cover (Table 3.2). Final bearing year net ramet populations were similar in both blueberry and bare soil patches at Purdy and Wyvern, though a significant difference in
final bearing year net ramet density did persist between quadrat cover at Pigeon Hill (Table 3.2). Bearing year $R_o$ values in both blueberry and bare soil patches were less than 1 and did not vary significantly (Table 3.2).

The initial ANOVA on production cycle $R_o$ values indicated significant quadrat cover (p<0.0001) and site (p=0.0044) effects, so data are presented separately for each quadrat cover at each site. Production cycle $R_o$ values were significantly higher in blueberry than in bare soil patches at Purdy and Pigeon Hill, but not Wyvern (Table 3.3).

The total density of new seedlings emerging, percent seedling survival, and final net seedling populations were generally similar across blueberry and bare soil patches at each site (Table 3.4). The total density of seedlings emerged varied across sites and ranged from 25 ± 42 to 619 ± 121 (mean ± SE) seedlings m$^{-2}$ (Table 3.4). Only at Pigeon Hill in the crop year did the density of new seedlings emerging vary across each type of quadrat cover, with a higher density of seedlings emerging in blueberry than in bare soil patches (Table 3.4). Therefore all new, dead, and net seedling data were combined across quadrat cover at each site to simplify data presentation. Season-long seedling emergence was observed at most sites, though a distinct early season flush of seedlings occurred at Pigeon Hill in 2011 (Fig. 3.3). Seedlings were subject to mortality almost immediately following the onset of emergence (Fig. 3.3) and seedling survival rates ranged from 6 ± 6 to 51 ± 12 % (Table 3.4).
Table 3.2. Analysis of demographic parameters for red sorrel ramet populations in blueberry and bare soil patches in three commercial lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Production Year</th>
<th>Quadrat Location</th>
<th>Mean Initial Ramet Density</th>
<th>Mean Density of New Ramets Emerged</th>
<th>Mean Ramet mortality</th>
<th>Mean Final Net Ramet Density</th>
<th>Single-Season Ro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdy-2009</td>
<td>Non-bearing</td>
<td>Blueberry Patch</td>
<td>317 ± 117a²</td>
<td>994 ± 124a</td>
<td>30 ± 5a</td>
<td>917 ± 87a</td>
<td>4.8 ± 1.25a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil Patch</td>
<td>531 ± 117a</td>
<td>803 ± 124a</td>
<td>56 ± 5b</td>
<td>597 ± 87b</td>
<td>1.3 ± 1.25a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purdy-2010</td>
<td>Bearing</td>
<td>Blueberry Patch</td>
<td>833 ± 88a</td>
<td>475 ± 139a</td>
<td>63 ± 11a</td>
<td>567 ± 223a</td>
<td>0.6 ± 0.21a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil Patch</td>
<td>542 ± 88a</td>
<td>361 ± 139a</td>
<td>80 ± 11a</td>
<td>222 ± 223a</td>
<td>0.3 ± 0.21a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyvern-2009</td>
<td>Non-bearing</td>
<td>Blueberry Patch</td>
<td>15 ± 2a (218)</td>
<td>1381 ± 111a</td>
<td>29 ± 5a</td>
<td>1147 ± 131a</td>
<td>5.4 ± 0.7a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil Patch</td>
<td>22 ± 2a (491)</td>
<td>994 ± 111b</td>
<td>55 ± 5b</td>
<td>667 ± 131b</td>
<td>1.5 ± 0.7b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyvern-2010</td>
<td>Bearing</td>
<td>Blueberry Patch</td>
<td>1075 ± 180a</td>
<td>1461 ± 134a</td>
<td>82 ± 7a</td>
<td>408 ± 110a</td>
<td>0.5 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil Patch</td>
<td>611 ± 180a</td>
<td>836 ± 134a</td>
<td>66 ± 7a</td>
<td>477 ± 110a</td>
<td>0.8 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon Hill-</td>
<td>Non-bearing</td>
<td>Blueberry Patch</td>
<td>575 ± 62a</td>
<td>2252 ± 214a</td>
<td>20 ± 2a</td>
<td>2275 ± 184a</td>
<td>4.0 ± 0.3a</td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td>Bare Soil Patch</td>
<td>725 ± 62a</td>
<td>1450 ± 214b</td>
<td>40 ± 2b</td>
<td>1305 ± 184b</td>
<td>1.8 ± 0.3b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon Hill-</td>
<td>Bearing</td>
<td>Blueberry Patch</td>
<td>1694 ± 137a</td>
<td>1605 ± 107a</td>
<td>52 ± 3a</td>
<td>39 ± 2a</td>
<td>0.9 ± 0.1a</td>
</tr>
<tr>
<td>2011</td>
<td></td>
<td>Bare Soil Patch</td>
<td>1114 ± 137b</td>
<td>669 ± 107b</td>
<td>53 ± 3a</td>
<td>29 ± 2b (846)</td>
<td>0.8 ± 0.1a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

²Means followed by the same letter do not differ significantly according to a t-test of the difference between means at the 0.05 significance level.
Table 3.3. Mean production cycle $Ro$ values for red sorrel ramet populations in blueberry and bare soil patches in three lowbush blueberry fields in Nova Scotia, Canada. Production cycle $Ro$ values for Purdy and Wyvern were LOG(X) transformed to meet the assumptions of the variance analysis, and back-transformed means are presented in parentheses.

<table>
<thead>
<tr>
<th>Quadrat Location</th>
<th>Purdy</th>
<th>Wyvern</th>
<th>Pigeon Hill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry Patches</td>
<td>$0.4 \pm 0.30a^z$ (1.5)</td>
<td>$2.2 \pm 0.67a$ (2.7)</td>
<td>$1.0 \pm 0.15a$ (0.30)</td>
</tr>
<tr>
<td>Bare Soil Patches</td>
<td>$-1.2 \pm 0.30b$ (0.30)</td>
<td>$1.3 \pm 0.67a$ (1.2)</td>
<td>$0.2 \pm 0.15b$ (1.2)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>$p = 0.0090$</td>
<td>$p = 0.3519$</td>
<td>$p = 0.0076$</td>
</tr>
</tbody>
</table>

*Mean $Ro$ values for each site followed by the same letter do not differ significantly according to a t-test ($\alpha = 0.05$).

Table 3.4. Mean total density, percent survival, and net population density of red sorrel seedlings emerging in blueberry and bare soil patches in four lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Quadrat Location</th>
<th>Total Seedlings Emerged</th>
<th>Seedling Survival</th>
<th>Net Seedling Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdy-2010</td>
<td>Blueberry Patch</td>
<td>$50 \pm 27a^z$</td>
<td>$23 \pm 10a$</td>
<td>$8 \pm 15a$</td>
</tr>
<tr>
<td></td>
<td>Bare Soil Patch</td>
<td>$58 \pm 27a$</td>
<td>$51 \pm 12a$</td>
<td>$31 \pm 15a$</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>$p = 0.8356$</td>
<td>$p = 0.1259$</td>
<td>$p = 0.3235$</td>
</tr>
<tr>
<td>Wyvern-2010</td>
<td>Blueberry Patch</td>
<td>$25 \pm 42a$</td>
<td>$7 \pm 8a$</td>
<td>$3 \pm 10a$</td>
</tr>
<tr>
<td></td>
<td>Bare Soil Patch</td>
<td>$125 \pm 42a$</td>
<td>$10 \pm 7a$</td>
<td>$19 \pm 10a$</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>$p = 0.1421$</td>
<td>$p = 0.7848$</td>
<td>$p = 0.2605$</td>
</tr>
<tr>
<td>Pigeon Hill-2010</td>
<td>Blueberry Patch</td>
<td>$233 \pm 33a$</td>
<td>$21 \pm 8a$</td>
<td>$47 \pm 17a$</td>
</tr>
<tr>
<td></td>
<td>Bare Soil Patch</td>
<td>$156 \pm 33a$</td>
<td>$29 \pm 8a$</td>
<td>$50 \pm 17a$</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>$p = 0.1421$</td>
<td>$p = 0.5378$</td>
<td>$p = 0.9130$</td>
</tr>
<tr>
<td>Pigeon Hill-2011</td>
<td>Blueberry Patch</td>
<td>$619 \pm 121a$</td>
<td>$6 \pm 6a$</td>
<td>$39 \pm 12a$</td>
</tr>
<tr>
<td></td>
<td>Bare Soil Patch</td>
<td>$125 \pm 121b$</td>
<td>$15 \pm 6a$</td>
<td>$14 \pm 12a$</td>
</tr>
<tr>
<td></td>
<td><em>p</em>-value</td>
<td>$p = 0.0279$</td>
<td>$p = 0.3134$</td>
<td>$p = 0.2029$</td>
</tr>
</tbody>
</table>

*Mean values for each site and quadrat cover followed by the same letter do not differ significantly according to a t-test ($\alpha = 0.05$).
3.5.2. Red Sorrel Root Depth

There were no significant site X core depth (p = 0.3832) or site X core location (p = 0.4798) interaction effects on root biomass, but there was a significant depth X core location interaction (p=0.0196). Root biomass data for each core location were therefore combined across sites and analyzed separately. The majority of the root biomass (>80%) was in the top 0-7cm in both blueberry and bare soil patches (Table 3.5). More root biomass was found in the 7-14 than in the 14-21cm core depth in blueberry patches, but root biomass was similar between these depths in bare soil patches (Table 3.5).
Table 3.5. Mean red sorrel root biomass at three core depths in blueberry and bare soil patches in three lowbush blueberry fields in Nova Scotia, Canada. Root biomass values were LOG(X) transformed to meet the assumptions of the variance analysis, and back-transformed means are presented in parentheses.

<table>
<thead>
<tr>
<th>Core Depth</th>
<th>Core Sample Location</th>
<th>Blueberry Patch</th>
<th>Bare Soil Patch</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7 cm</td>
<td></td>
<td>-0.1543 ± 0.2238a (0.86)</td>
<td>0.2207 ± 0.1616a (1.25)</td>
</tr>
<tr>
<td>7-14 cm</td>
<td></td>
<td>-2.3125 ± 0.2238b (0.10)</td>
<td>-2.7758 ± 0.1570b (0.06)</td>
</tr>
<tr>
<td>14-21 cm</td>
<td></td>
<td>-3.3435 ± 0.2238c (0.04)</td>
<td>-2.9643 ± 0.1570b (0.05)</td>
</tr>
</tbody>
</table>

Core volume of 331 cm³

Means followed by the same letter do not differ significantly according to a Tukey’s means comparison at alpha = 0.05.

3.5.3. Ramet and Seedling Phenological Development

No seedlings flowered in the year of emergence. The percent contribution of each ramet cohort to the total population of flowering ramets was analyzed separately at each site as violations in the assumptions for the ANOVA in the combined data set could not be remedied through data transformation. There was a significant ramet cohort X quadrat cover interaction at Wyvern-2009 (p=0.0036) and Pigeon Hill-2011 (p=0.0033), so data are presented separately for each type of quadrat cover at each site to simplify data presentation (Tables 3.6 and 3.7). The percent contribution of ramets to the total population of flowering ramets varied significantly with ramet cohort at each site (p<0.0001). The majority of ramets that flowered (>70%) were from the overwintering cohort in both blueberry and bare soil patches (Tables 3.6 and 3.7). New ramets emerging in May and June contributed similar proportions of ramets to the flowering population at most sites, but no new ramets emerging between July and November flowered in the year of emergence (Tables 3.6 and 3.7). Although the majority of flowering ramets were from the overwintering cohort, the proportion of ramets within this cohort that actually flowered was variable and ranged from 39-75% (Table 3.8). While the majority of overwintering ramet
populations (58%) were significantly segregated to flowering ramets ($X^2 \geq 24.8; p<0.0001$), 33% segregated evenly between flowering and vegetative ramets ($X^2 \leq 3.5; p\geq 0.0602$) and 8% segregated significantly to vegetative ramets ($X^2 = 54.8; p<0.0001$) (Table 3.8).

Table 3.6. Percentage contribution of red sorrel ramet cohorts to the total number of flowering ramets in blueberry patches at three non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Month of Emergence</th>
<th>% Contribution to Total Number of Flowering Ramets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwinter</td>
<td>88 ± 5.4a$^z$</td>
</tr>
<tr>
<td>May</td>
<td>5 ± 5.4b</td>
</tr>
<tr>
<td>June</td>
<td>7 ± 5.4b</td>
</tr>
<tr>
<td>July</td>
<td>0$^y$</td>
</tr>
<tr>
<td>August</td>
<td>0</td>
</tr>
<tr>
<td>September</td>
<td>0</td>
</tr>
<tr>
<td>October / November</td>
<td>0</td>
</tr>
</tbody>
</table>

$^z$Means followed by the same letter where three comparisons are made do not differ significantly according to a Tukey’s means comparison test at alpha=0.05; means followed by the same letter where only two means are compared do not differ significantly according to a t-test of the difference between the means at an alpha level of 0.05.

$^y$Cohorts that did not contribute any flowering ramets to the total population of flowering ramets were not included in means comparisons.
Table 3.7. Percentage contribution of red sorrel ramet cohorts to the total number of flowering ramets in bare soil patches at three non-bearing and three bearing-year lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Month of Emergence</th>
<th>% Contribution to Total Number of Flowering Ramets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwinter</td>
<td>90 ± 1.4a</td>
</tr>
<tr>
<td>May</td>
<td>9 ± 1.4b</td>
</tr>
<tr>
<td>June</td>
<td>1 ± 1.4b</td>
</tr>
<tr>
<td>July</td>
<td>0</td>
</tr>
<tr>
<td>August</td>
<td>0</td>
</tr>
<tr>
<td>September</td>
<td>0</td>
</tr>
<tr>
<td>October / November</td>
<td>0</td>
</tr>
</tbody>
</table>

*Means followed by the same letter where three comparisons are made do not differ significantly according to a Tukey’s means comparison test at alpha=0.05; means followed by the same letter where only two means are compared do not differ significantly according to a t-test of the difference between the means at an alpha level of 0.05.

*Cohorts that did not contribute any flowering ramets to the total population of flowering ramets were not included in means comparisons.*
Table 3.8. Mean density and test of 50:50 segregation of flowering and vegetative ramets in overwintering red sorrel ramet populations in blueberry and bare soil patches in three commercial lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Production Year</th>
<th>Quadrat Location</th>
<th>Mean Overwintering Ramet Density</th>
<th>Mean Density of Overwintering Ramets Flowering</th>
<th>Mean Density of Overwintering Ramets Remaining Vegetative</th>
<th>$-X^2-$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdy-2009</td>
<td>Non-bearing</td>
<td>Blueberry</td>
<td>317 ± 118</td>
<td>161 ± 63</td>
<td>156 ± 100</td>
<td>0.1</td>
<td>0.7788</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil</td>
<td>531 ± 117</td>
<td>372 ± 73</td>
<td>158 ± 71</td>
<td>86.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Purdy-2010</td>
<td>Bearing</td>
<td>Blueberry</td>
<td>833 ± 87</td>
<td>536 ± 174</td>
<td>297 ± 128</td>
<td>68.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil</td>
<td>542 ± 90</td>
<td>292 ± 108</td>
<td>250 ± 34</td>
<td>3.3</td>
<td>0.0712</td>
</tr>
<tr>
<td>Wyvern-2009</td>
<td>Non-bearing</td>
<td>Blueberry</td>
<td>222 ± 33a</td>
<td>125 ± 33</td>
<td>97 ± 36</td>
<td>3.5</td>
<td>0.0602</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil</td>
<td>519 ± 138</td>
<td>367 ± 116</td>
<td>153 ± 37</td>
<td>88.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wyvern-2010</td>
<td>Bearing</td>
<td>Blueberry</td>
<td>1075 ± 244</td>
<td>650 ± 229</td>
<td>425 ± 39</td>
<td>47.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil</td>
<td>611 ± 71</td>
<td>367 ± 47</td>
<td>244 ± 70</td>
<td>24.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pigeon Hill-2010</td>
<td>Non-bearing</td>
<td>Blueberry</td>
<td>575 ± 58</td>
<td>403 ± 43</td>
<td>172 ± 79</td>
<td>92.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil</td>
<td>725 ± 68</td>
<td>544 ± 89</td>
<td>181 ± 52</td>
<td>181.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pigeon Hill-2011</td>
<td>Bearing</td>
<td>Blueberry</td>
<td>1694 ± 186</td>
<td>831 ± 93</td>
<td>864 ± 220</td>
<td>0.6</td>
<td>0.4228</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bare Soil</td>
<td>1114 ± 56</td>
<td>433 ± 24</td>
<td>680 ± 51</td>
<td>54.8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Ramets with open flowers were first observed at each site around day of year 140 (Fig. 3.4). Male and female ramets generally flowered at the same time, and peak flowering occurred by day 180 to 200 of each year (Fig. 3.5). Female ramets set seed almost immediately after the first open flowers were observed, and peak seed set generally coincided with peak flowering (Fig. 3.5). Flowering ramet populations at Wyvern and Pigeon Hill did not deviate from a 1:1 sex ratio (Table 3.9). In contrast, flowering ramet populations at Purdy were dominated by female ramets in the non-bearing year but by male ramets in the bearing year (Table 3.9).

3.5.4. Ramet Cohort Survival, Population Age Structure, and Life-Cycle Model

Survival of overwintering ramets ranged from 3 to 55 and 9 to 46% in blueberry and bare soil patches, respectively (Tables 3.10 and 3.11). Mortality was generally higher and began earlier in the season for overwintering ramets that remained vegetative compared to those that flowered (Figs. 3.6 and 3.7). Survival of vegetative overwintering ramets ranged from 0 to 30% in blueberry patches and 5 to 51% in bare soil patches while survival of flowering ramets ranged from 7 to 37% and 35 to 40% in blueberry and bare soil patches, respectively (Figs. 3.6 and 3.7). Survival of new ramets, when grouped by month of emergence, was variable and ranged from 11 to 95 and 17 to 94% in blueberry and bare soil patches, respectively (Tables 3.10 and 3.11).

There was a significant ramet cohort X quadrat cover interaction effect on the percent contribution of each cohort to final net ramet populations at all sites (p ≤ 0.0162) except Wyvern (p=0.0990). The age structure of final net ramet populations was therefore presented separately for blueberry and bare soil patches at each site (Fig. 3.7). The initial overwintering ramet population comprised anywhere from 8 to 34 and 17 to 59% of final net ramet populations in
Table 3.9. Sex ratio of flowering red sorrel ramets in three non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Production Year</th>
<th>Total Male Ramets Counted</th>
<th>Total Female Ramets Counted</th>
<th>Total</th>
<th>(X^2)</th>
<th>Yates Corrected (X^2)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdy-2009</td>
<td>Non-bearing</td>
<td>60</td>
<td>136</td>
<td>196</td>
<td>---</td>
<td>28.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Purdy-2010</td>
<td>Bearing</td>
<td>184</td>
<td>117</td>
<td>301</td>
<td>14.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyvern-2009</td>
<td>Non-bearing</td>
<td>98</td>
<td>89</td>
<td>187</td>
<td>---</td>
<td>0.3</td>
<td>0.5585</td>
</tr>
<tr>
<td>Wyvern-2010</td>
<td>Bearing</td>
<td>186</td>
<td>200</td>
<td>386</td>
<td>0.5</td>
<td></td>
<td>0.4761</td>
</tr>
<tr>
<td>Pigeon Hill-2010</td>
<td>Non-bearing</td>
<td>171</td>
<td>205</td>
<td>376</td>
<td>3.1</td>
<td></td>
<td>0.0795</td>
</tr>
<tr>
<td>Pigeon Hill-2011</td>
<td>Bearing</td>
<td>246</td>
<td>243</td>
<td>489</td>
<td>0.02</td>
<td></td>
<td>0.8921</td>
</tr>
</tbody>
</table>

\(^2\)Yates Corrected \(X^2\) used for all analyses in which the total number of flowering ramets was <200

Table 3.10. Mean density and percent survival of monthly red sorrel ramet cohorts in blueberry patches at one non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Ramet Cohort</th>
<th>Purdy-2010</th>
<th>Wyvern-2010</th>
<th>Pigeon Hill-2010</th>
<th>Pigeon Hill-2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Density of New Ramets Produced</td>
<td>Mean Survival</td>
<td>Mean Density of New Ramets Produced</td>
<td>Mean Survival</td>
</tr>
<tr>
<td>Overwinter</td>
<td>833 ± 87</td>
<td>27 ± 14</td>
<td>1075 ± 244</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>May</td>
<td>117 ± 17</td>
<td>46 ± 20</td>
<td>197± 39</td>
<td>26 ± 10</td>
</tr>
<tr>
<td>June</td>
<td>53 ± 32</td>
<td>40 ± 23</td>
<td>144 ± 79</td>
<td>32 ± 24</td>
</tr>
<tr>
<td>July</td>
<td>128 ± 40</td>
<td>62 ± 11</td>
<td>547 ± 121</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>August</td>
<td>97 ± 65</td>
<td>36 ± 21</td>
<td>225 ± 40</td>
<td>32 ± 15</td>
</tr>
<tr>
<td>September</td>
<td>67 ± 23</td>
<td>89 ± 7</td>
<td>283 ± 92</td>
<td>40 ± 18</td>
</tr>
<tr>
<td>October / November</td>
<td>14 ± 7</td>
<td>75 ± 25</td>
<td>53 ± 17</td>
<td>67 ± 24</td>
</tr>
</tbody>
</table>
Table 3.11. Mean density and percent survival of monthly red sorrel ramet cohorts in bare soil patches at one non-bearing and three bearing year lowbush blueberry fields in Nova Scotia, Canada.

<table>
<thead>
<tr>
<th>Ramet Cohort</th>
<th>Purdy-2010</th>
<th>Wyvern-2010</th>
<th>Pigeon Hill-2010</th>
<th>Pigeon Hill-2011</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Density of New Ramets Produced</td>
<td>Mean Survival</td>
<td>Mean Density of New Ramets Produced</td>
<td>Mean Survival</td>
</tr>
<tr>
<td>Overwinter</td>
<td>542 ± 90</td>
<td>9 ± 6</td>
<td>611 ± 71</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>May</td>
<td>169 ± 60</td>
<td>54 ± 5</td>
<td>344 ± 21</td>
<td>21 ± 7</td>
</tr>
<tr>
<td>June</td>
<td>100 ± 27</td>
<td>20 ± 8</td>
<td>175 ± 74</td>
<td>32 ± 14</td>
</tr>
<tr>
<td>July</td>
<td>28 ± 7</td>
<td>56 ± 26</td>
<td>172 ± 23</td>
<td>58 ± 13</td>
</tr>
<tr>
<td>August</td>
<td>42 ± 22</td>
<td>26 ± 16</td>
<td>56 ± 12</td>
<td>74 ± 12</td>
</tr>
<tr>
<td>September</td>
<td>17 ± 17</td>
<td>17 ± 17</td>
<td>64 ± 19</td>
<td>60 ± 15</td>
</tr>
<tr>
<td>October / November</td>
<td>8 ± 5</td>
<td>38 ± 24</td>
<td>25 ± 8</td>
<td>94 ± 6</td>
</tr>
</tbody>
</table>
Fig. 3.5. Single-season depletion curves of flowering and vegetative overwintering red sorrel ramets in blueberry patches at A) Purdy-2010, B) Wyvern-2010, C) Pigeon Hill- 2010, and D) Pigeon Hill-2011.
Fig. 3.6. Single-season depletion curves of flowering and vegetative overwintering red sorrel ramets in bare soil patches at A) Purdy-2010, B) Wyvern-2010, C) Pigeon Hill- 2010, and D) Pigeon Hill-2011.
Fig. 3.7. Mean percent contribution of overwintering and monthly ramet cohorts to the final net ramet population in blueberry and bare soil patches at A) Purdy-2010, B) Wyvern-2010, C) Pigeon Hill-2010, and D) Pigeon Hill-2011. Error bars represent 1 standard error of the mean.
Fig. 3.8. Life-cycle diagram describing the development and survival of red sorrel ramets and seedlings in non-bearing and bearing year lowbush blueberry fields in Nova Scotia, Canada. Transition probabilities for all stages of ramet development and seedling recruitment were determined directly from field census data collected from 3 lowbush blueberry fields in Nova Scotia, Canada. Where two transition probabilities are indicated, significantly different transition probabilities for ramet populations in bare soil patches are provided in parentheses.
blueberry and bare soil patches, respectively, at each site (Fig. 3.7). The proportion of monthly ramet cohorts that comprised final net ramet populations at each site was variable and ranged from 3 to 27 and 2 to 51% in blueberry and bare soil patches, respectively (Fig. 3.7).

Based on the transition probabilities obtained from the field data, a life-cycle model for red sorrel over the 2-year lowbush blueberry production cycle was proposed (Fig. 3.8). There was a significant effect of quadrat cover (p=0.0085) but no significant quadrat cover X site interaction (p=0.7504) effect on the probability of new ramets remaining vegetative or flowering in the non-bearing year. Therefore, transition probabilities for new ramets flowering or remaining vegetative for each type of quadrat cover were combined across sites. The majority of new ramets (>95%) remained vegetative in the non-bearing year, though small significant differences in transition probabilities between blueberry and bare soil patches were found for new ramets remaining vegetative or flowering (Fig. 3.8). Mean % survival of new vegetative ramets was significantly higher in blueberry than in bare soil patches (p=0.004), but survival of new flowering ramets was similar across both types of quadrat cover (Fig. 3.8). There was no significant quadrat cover (p≥0.3413) or site X quadrat cover (p≥0.8721) interaction effect on the probability of overwintering ramets remaining vegetative or flowering in the non-bearing year (Fig. 3.8). Survival of overwintering ramets that remained vegetative was similar across both types of quadrat cover, but survival of overwintering ramets that flowered was higher in blueberry than in bare soil patches in the non-bearing year (p=0.0049)(Fig. 3.8).

There was no effect of site (p=0.4870), quadrat cover (p=0.5000), or the site X quadrat cover interaction (p=0.5701) on the probability of overwintering ramets flowering, overwintering
ramets remaining vegetative, or the survival of overwintering flowering or vegetative ramets in the bearing year (Fig. 3.8). When combined across sites, data for the probability of new ramets flowering or remaining vegetative in blueberry and bare soil patches in the bearing year could not be made to conform to the assumptions of the variance analysis. Therefore, t-tests of the difference between these probabilities for blueberry and bare soil patches were determined separately for each site. New ramets had a higher probability of flowering in bare soil than in blueberry patches in the bearing year at Pigeon Hill (p=0.0470; Fig. 3.8). The probability of new ramets flowering was similar in blueberry and bare soil patches at Purdy and Wyvern (p=0.7181) and averaged 1 ± 1 and 4 ± 3% (mean ± SE) at Purdy and Wyvern, respectively.

Survival of new vegetative ramets in the bearing year varied significantly across sites (p=0.0125) but not quadrat cover (p=0.4388). Survival of new vegetative ramets at Pigeon Hill and Purdy averaged 64 ± 6 and 50 ± 6% (mean ± SE) and did not differ according to a Tukey’s means comparison (p=0.2717). Survival of new ramets at Wyvern averaged 35 ± 6% and was significantly lower than survival observed at Pigeon Hill (p=0.0093) but not Purdy (p=0.2110). The transition probability for survival of new vegetative ramets in the bearing year for Pigeon Hill is presented (Fig. 3.8). Survival of new ramets that flowered did not vary significantly across sites (p=0.7990) or quadrat cover in the bearing year (p=0.4470)(Fig. 3.8).

The overwinter survival of net ramet populations (88 ± 3%) was assumed to be similar between the non-bearing and bearing year. Seedling survival, and therefore estimated recruitment of new genets, was based on an average across all sites and quadrat locations based on the results in Table 3.4.
3.6. Discussion

This study highlighted three main components of red sorrel populations in lowbush blueberry fields in Nova Scotia; the initial overwintering ramet population, the population of new ramets emerging within each season, and seedlings. The majority of the flowering population is comprised of ramets from the overwintering cohort (Tables 3.6 and 3.7), and surviving vegetative and flowering ramets from this cohort (Figs. 3.5 and 3.6) contribute to final net ramet populations at the end of each season (Fig. 3.7). New ramets emerge throughout the season (Figs. 3.1 and 3.2) with multiple cohorts of new ramets contributing to the final net ramet populations at each site (Fig. 3.7). The majority of these new ramets remain vegetative (Tables 3.6 and 3.7; Fig. 3.8), and appear to require an overwintering period for flower induction to occur. Seasonal recruitment of new seedlings into established red sorrel populations was observed at all sites and years in this study. Seedling survival ranged from 6 ± 6 to 51 ± 12% (Table 3.4) and averaged 19 ± 4% across sites. All seedlings remained vegetative in the year of emergence under field conditions.

Field populations of red sorrel are known to contain both flowering and vegetative ramets (Fujitaka and Sakai, 2007; Putwain and Harper, 1972), but the role of a distinct overwintering ramet population for flowering has not been previously reported. Kennedy (2009) indicated the potential presence of an overwintering red sorrel ramet population in lowbush blueberry fields, but the direct link between this portion of the ramet population and flowering was unclear. Although there was a significant cohort X quadrat cover interaction effect on the contribution of each cohort to the flowering ramet population, the majority of flowering ramets (>70%) were
from the overwintering cohort in both blueberry and bare soil patches (Tables 3.6 and 3.7). Flowering is also confined to overwintering ramets in the sedge species Carex arenaria (Noble et al., 1979), but very few clonal plants have actually been documented to exhibit this behavior. No reports of a vernalization requirement for flower induction in red sorrel could be found in the literature, but Harris (1970) has indicated that low temperatures may be required for flowering in some red sorrel populations. The lack of flowering observed in established seedlings during the year of emergence would support this idea. However, seedlings of other clonal plants not requiring vernalization often only flower in the second year after emergence or later under field conditions (Baskin and Baskin, 1988; Moore, 1947). The majority of new ramets emerging at each site (>95%, Fig. 3.8), however, remained vegetative as well and apparently require exposure to some type of stimulus to induce flowering under field conditions.

Cook (1985) has suggested that maintenance of both vegetative and flowering ramets at the genet level in clonal plants is likely controlled through flower induction via environmental stimuli such as photoperiod or vernalization, but very little work in this area has been conducted with clonal plants. A balance between vegetative and flowering ramets is essential for growth of individual genets as flowering ramets seldom export assimilates to support growth of the genet (Muir, 1995; Noble and Marshall, 1983; Ong et al., 1978; Pitelka and Ashmun, 1985). Red sorrel genets in lowbush blueberry fields in Nova Scotia appear to maintain the balance between vegetative and flowering ramets, and thus continual genet growth, through a vernalization requirement for flowering at the ramet level of organization. Ramets with open flowers were observed at each site between day 131 and 152 (May 11 – June 1) of each year (Fig. 3.4), which coincides with the onset of blueberry bloom (White et al., 2012; Chapter 2). Flowering red sorrel
ramets are also foraged by introduced pollinators such as honey bees (Hughes, 2012), though the impact of this foraging on blueberry pollination is unknown. The fact that flowering ramet populations are comprised primarily of ramets from the overwintering cohort provides opportunities for the development of weed management strategies designed to prevent the establishment of overwintering ramet populations, and thus reducing the density of flowering ramets in subsequent years.

The large proportion of the overwintering ramet population that remained vegetative (Table 3.8) seems most likely related to the age structure that was observed in net ramet populations at each site (Fig. 3.7), and the potential impacts of this age structure on ramet size or exposure to environmental conditions prior to vernalization. Based purely on observation, no obvious trends in ramet size or leaf number were detected in net ramet populations at the end of each season. However, it is likely that later emerging ramets had fewer leaves or were smaller than ramets emerging earlier in the season. Rosette size is an important factor affecting the vernalization response in many monocarpic perennials (Gross, 1981; Kachi and Hirose, 1983; Klinkhammer et al., 1987a), and ramet size has been associated with a higher probability of flowering in some clonal plants (Cain and Damman, 1997; Eriksson, 1988; Pitelka et al., 1985). Wellensiek (1964) has also demonstrated an increased flowering response following vernalization in plants with an abundance of young leaves, and thus an abundance of active cell division. In the conditions of our study it seems more likely that older ramets would have a greater abundance of younger leaves, and thus may have been more susceptible to flower induction through vernalization. The vernalization response in some species is also affected by pre-ernalization stimuli such as decreasing photoperiod and temperature. For example, Foley et
al. (2009) found that *Euphorbia esula* plants required exposure to decreasing temperatures prior to vernalization for flower induction to occur. Medd and Lovett (1978) found a reduced vernalization duration requirement for flower induction in *Carduus nutans* (L.) ssp. *nutans* when plants were grown in short photoperiods prior to cold treatment. Thus, exposure to appropriate environmental stimuli prior to vernalization can be as important as vernalization itself in terms of the induction of flowering. The role of pre-vernalterization stimuli in red sorrel ramets is unclear. The season-long ramet emergence and distinct age structure of net ramet populations, however, certainly indicates a net ramet population composed of individuals exposed to different extremes of environmental stimuli prior to vernalization. The potential influence of this phenomenon on flower induction warrants further investigation in order to understand the factors affecting red sorrel flowering competency in lowbush blueberry fields.

Factors that might induce flowering in new ramets emerging in May and June are less clear as very little work has been conducted to investigate the role of environmental conditions on the maintenance of vegetative and flowering ramets in field populations of red sorrel. The plant requires long days for flowering (Carlson, 1965) and has been reported to flower under long days and ambient greenhouse conditions (Lovett Doust and Lovett Doust, 1987; Zimmerman and Lechowicz, 1982). Therefore, some ramet populations in lowbush blueberry fields in Nova Scotia may originate from genets that do not require a cold period to induce flowering. However, durations of up to 140 days of growth under long days have been reported before flowering will occur (Bavrina et al., 1991). Therefore, ramets emerging in May and June in Nova Scotia likely have not been exposed to a sufficient duration of long days to induce flowering given that most of these ramets flowered within 14 to 68 days after emergence.
Bavrina et al. (1991) found that growth of red sorrel under short days prior to transfer to long days accelerates the flowering response. Exposure of new ramets to increasing photoperiod in early spring might therefore induce a flowering response if ramets emerge prior to a critical daylength. This type of flowering response occurs in tillers of timothy (*Phleum pretense*) emerging in early spring (Langer, 1956). Growth under short photoperiods prior to exposure to long days also acts as a substitute for vernalization in some species (Wellensiek, 1960). Exposure of new ramets to increasing photoperiod in early spring might therefore act as a substitution for vernalization in some red sorrel ramets requiring a cold treatment for flower induction. Finally, D’Aloia et al. (2008) reported an increased response to short durations of vernalization in *Sinapis alba* when plants vernalized for one week were exposed to long days immediately after vernalization. Extrapolating to field conditions, D’Aloia et al. (2008) speculate that exposure to short durations of cold concurrent with increasing daylengths in spring may satisfy the vernalization response in some species. Unfortunately, our current lack of understanding of the effects of photoperiod and vernalization on flowering of red sorrel ramets in lowbush blueberry limit interpretation of the results of this study.

Although purely speculative, a potential explanation for flowering of new ramets is the transfer of flowering competency from vernalized to unvernalized ramets (Sachs, 2008). Though mobility of the flowering stimulus induced by vernalization is variable across species where this has been studied, transmission of the stimulus through grafting has been demonstrated (Amasino, 2004, Chouard, 1960). Schwabe (1954) has demonstrated the movement of the stimulus from the vernalized shoot tip to lateral apices formed after the vernalization period in the chrysanthemum, and translocation of a floral stimulus between tillers of some temperate grasses has also been
reported (Havstad et al., 2004). Physiological integration between ramets in many clonal plants is well documented (Ashmun et al., 1982; Pitelka and Ashmun, 1985) and may provide the opportunity for similar translocation of a flower stimulus between interconnected ramets. Though movement of nutrients between red sorrel ramets is limited (Klimeš and Klimišová, 1999), movement of photoassimilates between ramets does occur (Harris, 1972) and indicates a certain level of physiological integration between ramets. Research into this potential aspect of the reproductive biology of red sorrel ramets will likely be facilitated by an improved understanding of the factors regulating flowering in this species.

Finally, it is possible that new ramets counted in May and June were the result of re-growth of ramets that were counted as dead in the previous season. Lovett Doust (1981) reported that about 8% of the *R. repens* ramets tracked in her study died back to ground level but resumed growth in the subsequent season. Elastics associated with dead ramets were removed in the current study; re-growth in the exact positions of previously established ramets could therefore not be determined. All new ramets counted during this study, however, were similar and generally had 1-3 leaves at the time of marking; no obvious re-growth from previously established ramets was observed. It could likely be expected that ramets emerging as re-growth from previously established ramets would emerge prior to ramets emerging from newly initiated buds on the creeping root system. However, new ramets that flowered emerged throughout May and June and it seems unlikely that ramets emerging in late May or early June would be establishing as re-growth from previously established ramets. Future work in which the position of dead ramets is retained in each quadrat would be needed to determine if re-growth of previously established ramets contributes to the population of flowering ramets.
Survival of overwintering red sorrel ramets that flowered ranged from 7-40% (Figs. 3.5 and 3.6), indicating that some red sorrel ramets might exhibit a polycarpic habit in lowbush blueberry fields. Polycarpic ramets have been reported in other clonal species (Araki and Ohara, 2008; Eriksson, 1988; Pitelka et al., 1985), and our data suggests that red sorrel ramets are also capable of exhibiting a polycarpic growth habit. This could be confirmed in the field using similar methodology to that outlined in the current study. It is unclear as to why overwintering ramets that flowered had a higher survival rate than those remaining vegetative, but similar behavior has been reported for ramets of *Asarum canadense* (Cain and Damman, 1997). Higher survival of flowering *Asarum canadense* ramets was attributed to the larger size of flowering ramets compared to vegetative ramets (Cain and Damman, 1997), though similar conclusions for red sorrel ramets cannot be drawn. It is also unclear as to why flowering ramets from the overwintering cohort suffered higher mortality in bare soil than in blueberry patches during the non-bearing year, though this likely contributed to the lower net ramet densities in bare soil patches at the end of the non-bearing year (Table 3.2). Regardless of survival across quadrat cover, ramets from the initial overwintering cohort continue to contribute to net ramet populations at the end of each season (Fig. 3.7) and likely survive for multiple seasons. Individual cohorts of ramets in populations of *Ranunculus repens* (Lovett Doust, 1981) and *Hieracium pilosella* (Bishop et al., 1978) have been found to slowly decline over a period of 1-3 years, and Sarukhan and Harper (1973) estimated about 30% of *R. repens* ramets in any given year to be 2 years of age or older. Red sorrel ramet populations would also seem to retain ramets exceeding 2 years of age, with established overwintering ramet populations undergoing the same type of decline over multiple years as reported for *R. repens* and *H. Pilosella*. Further research would need to extend across multiple blueberry production cycles to determine the true turnover
rate of established overwintering ramet populations. An improved stage classification of the overwintering ramet population should also be established and used to help identify trends in ramet size or age that affect survival.

Sex ratios of red sorrel ramets in natural populations are variable but seldom segregate on a 1:1 basis (Putwain and Harper, 1972), so it is unclear why a 1:1 ratio of male to female ramets was found at Wyvern and Pigeon Hill (Table 3.9). Many populations migrate toward dominance of the population by either male (Escarré and Houssard, 1991) or female (Lovett Doust and Lovett Doust, 1987) ramets over time, primarily due to sex-related differences in response to environmental conditions or biomass allocation (Fujitaka and Sakai, 2007; Houssard et al., 1994). The change from a female- to male-biased population in the bearing year at the Purdy site may be indicative of the male-biased sex ratios that prevail in older ramet populations (Escarré and Houssard, 1991). The stability in the ratio of male and female ramets across both the non-bearing and bearing year at Wyvern and Pigeon Hill, however, would suggest that sex ratios of 1:1 might be more common in lowbush blueberry fields than other environments where red sorrel populations have been studied. Sex-specific responses of established red sorrel populations to environmental conditions in Nova Scotia are unknown, and differences in biomass allocation between established male and female genets in lowbush blueberry fields have not been confirmed. Our data on the survival of overwintering ramets indicate that many ramets persist in established populations for at least 2 years. If this pattern were to vary between ramets of male and female genets than respective changes in the sex ratio of ramets in established populations could be expected. Future work should focus specifically on the fate of male and female ramets
after flowering in an effort to generate data that can be used to test new hypotheses regarding factors regulating the sex ratios of red sorrel ramet populations in lowbush blueberry fields.

The overall flux in ramet populations at each site (Figs. 3.1 and 3.2) was regulated by relatively consistent cycles of ramet birth and death similar to those reported for ramets of other clonal species (Bishop et al., 1978; Lovett Doust, 1981; Noble et al., 1979). However, season-long red sorrel ramet emergence occurred in concert with a decline in the initial overwintering ramet population. Thus, as the overwintering ramet cohort undergoes its developmental fate and decline throughout the season, the overall net ramet population is replenished via season-long emergence of new ramets. With the exception of the study on C. arenaria (Noble et al., 1979), no other studies on ramet demography have identified this behavior in clonal plants. This overall flux in ramet populations was the primary aspect of red sorrel population biology affected by growth in blueberry or bare soil patches. Ramet dynamics of other clonal species have been shown to vary in contrasting habitats (Bishop et al., 1978; LovettDou, 1981; Navas and Garnier, 1990), but few of these studies have been conducted in an agricultural setting where differences in ramet dynamics have direct impacts on crop production. Fewer ramets emerged in bare soil patches at Wyvern and Pigeon Hill, and ramet mortality was higher in bare soil patches during the non-bearing year at all three study sites (Table 3.2). The higher ramet mortality in bare soil patches in the non-bearing year was associated with higher mortality of overwintering ramets that flowered and new ramets that remained vegetative (Fig. 3.8). Although only based on data from the Pigeon Hill site, overall net changes in ramet populations in the non-bearing year were similar within each type of quadrat cover across sites (Figs. 3.1 and 3.2). This resulted in larger final net ramet populations in blueberry patches in the non-bearing year (Table 3.2) and
subsequently higher density of flowering ramets in the bearing year (Table 3.8). Timing of red sorrel flowering appears to coincide with blueberry bloom (White et al., 2012; Chapter 2) and flowers are actively foraged by introduced pollinators (Hughes, 2012). Therefore, the large net gain to ramet populations in blueberry patches in the non-bearing year, combined with the apparent requirement for vernalization for flower induction, has potential impacts on blueberry yield irrespective of direct competition between the two species. Management strategies for the non-bearing year should therefore be primarily focused on increasing ramet mortality and reducing the size of the final net ramet population that in turn contributes to the flowering ramet population in the subsequent bearing year.

Cook (1985) stated that seasonal ramet mortality varies across years but that net ramet populations tend to remain stable over time. Mortality of red sorrel ramets was consistently lower in blueberry patches in the non-bearing year but similar across both blueberry and bare soil patches in the bearing year (Table 3.2; Figs. 3.1 and 3.2). Therefore, mortality of red sorrel ramets appears to follow a predictable pattern in lowbush blueberry fields during the 2-year production cycle. It is, however, not entirely clear why mortality was lower in blueberry patches in the non-bearing year. The majority of root biomass in both blueberry and bare soil patches was in the upper 0-7cm (Table 3.5). The primary difference at this soil depth across both types of quadrat cover is the presence of a surface organic layer extending to a depth of 2 to 4 cm in the blueberry patches (Penney et al., 1997). The maintenance of this surface organic layer is known to increase the vegetative growth of lowbush blueberry (Hicklenton et al., 2000a; Kender and Eggert, 1966), though little attention has been given to the potential role of this surface organic layer on the growth of perennial weeds in lowbush blueberry fields. Similar surface organic
layers in forest ecosystems tend to have higher concentrations of nutrients such as nitrogen and phosphorous when compared to the mineral soils located beneath (Smith et al., 1998). These nutrients are released during decomposition of the organic layer (Melillo et al., 1989; Moore et al., 2006) and thus become available to plants. Red sorrel produces more ramets in nutrient-rich than in nutrient-poor soils under controlled conditions (Klimeš and Klimešová, 1999), and higher survival rates of red sorrel have been reported for plants growing in the surface organic horizon when compared to lower mineral horizons (Ernst and Nelissen, 1979). This difference might disappear in the bearing year due to the large density of flowering ramets in blueberry patches (Table 3.8). As previously stated, flowering ramets in many clonal species seldom export assimilates to maintain growth of the genet (Noble and Marshall, 1983; Ong et al., 1978; Pitelka and Ashmun, 1985). High densities of flowering ramets may therefore result in there being limited resources available to maintain new vegetative ramets. Higher ramet density in the bearing year may also impact mortality through direct competition between ramets of different genets as clonal plants do not produce ramets at densities high enough for self-thinning to occur (Silvertown, 1982). It will therefore be important in future studies to determine the level of genet diversity in established red sorrel populations in lowbush blueberry fields if the impacts of intra-specific competition on ramet dynamics are to be understood.

With regards to stability of ramet populations over time, production cycle $Ro$ values for blueberry patches ranged from 1.5 to 2.7 (Table 3.3), indicating an increase in ramet populations between the spring of the non-bearing year and the autumn of the bearing year. In contrast, production cycle $Ro$ values for ramet populations in bare soil patches ranged from 0.3 to 1.3 (Table 3.3) and most closely resembled the stability in ramet populations over time described by
Cook (1985). Similar measures of ramet population growth over time for other species vary, but generally range from 0 to 2 for most species studied for durations of 2 to 3 years (Lovett Doust, 1981; Navas and Garnier, 1990; Newell et al., 1981; Pitelka et al., 1985; Sarukhan and Harper, 1973). Red sorrel ramet populations in blueberry patches therefore appear to have a higher propensity for population growth when compared to other clonal perennial species. This is likely related to the large single-season $Ro$ values associated with ramet populations in blueberry patches during the non-bearing year (Table 3.2). With the exception of a vineyard population of *Rubia peregrina* ramets reported by Navas and Garnier (1990), few net ramet populations of herbaceous perennials exhibit such high rates of growth. While stability of net ramet populations is usually stressed over the long term (Cook 1985), large changes in net ramet populations over the short term are important in the context of an agricultural system managed on a short term basis. The consistency of this large increase in net ramet populations across sites, however, needs to be noted as large changes in ramet populations over the short term are likely to have greater impacts on lowbush blueberry production than smaller changes in ramet populations over the long term.

Seedling recruitment in established populations of clonal plants ranges from fairly frequent (Coelho et al., 2008; Newell et al., 1981) to rare (Lovett Doust, 1981; Navas and Garnier, 1990) to completely absent (Bishop et al., 1978; Dickerman and Wetzel, 1985; Hartnett and Bazazz, 1985a; Pitelka et al., 1985). Establishment from seed is generally rare or completely absent in established populations of red sorrel (Putwain et al., 1968; Putwain and Harper, 1970; Kennedy, 2009), despite rather high seed production in most populations (Escarré and Thompson, 1991; Kennedy, 2009). Seedling establishment was also reported to be less than 2%
in an early successional environment in which the seedbank was dominated by red sorrel (Marteinsdóttir et al., 2010). Our results however, contradict this and indicate that establishment of new genets from seed likely occurs on a regular basis in lowbush blueberry fields. Although seedling survival was variable across sites (Table 3.4), the results are quite significant with regards to the maintenance of genetic diversity in established populations. Eriksson (1989) stated that populations of clonal plants are subject to either initial or repeated seedling recruitment. Initial seedling recruitment is the result of seed germination due to a rare disturbance and generally results in low genetic diversity in established populations (Eriksson, 1989). In contrast, populations subject to repeated seedling recruitment, as seems the case for red sorrel populations in lowbush blueberry, recruit new genets from seed on a regular basis and therefore maintain genetically diverse populations (Coelho et al., 2008; Eriksson, 1989; Soane and Watkinson, 1979). Eriksson (1993) maintains that these two categories should be viewed as endpoints on a continuum of seedling recruitment in clonal plants as the density of established genets tends to reduce seedling establishment (Waite and Hutchings, 1979). Our data were collected without the influence of common agricultural inputs such as herbicides and fertilizers in an attempt to establish a baseline understanding of seedling recruitment into established populations. The most immediate impact of a successful management strategy for red sorrel will be a reduction in genet density. The impacts of reducing genet density, in combination with other agronomic practices, on seedling recruitment needs to be considered for management strategies to be truly successful in the long term. Red sorrel seeds are also readily dispersed by blueberry harvesters (Boyd and White, 2009), and the impacts of this dispersal on the genetic diversity of established populations may be underappreciated.
The emergence pattern of seedlings is also important to consider with regards to the potential for seedlings to become successfully established. With the exception of the Pigeon Hill site in 2011, seedlings emerged throughout the season over a period of 144 to 197 days. Weed control with soil applied herbicides prior to blueberry emergence in the spring of the non-bearing year is one of the primary components of weed management in lowbush blueberry. The most common herbicide applied in this manner is hexazinone, which is generally applied in late April or early May prior to blueberry emergence. Hexazinone tends to dissipate rapidly in blueberry soils however, with less than 10% of applied hexazinone remaining 60 days after application (Jensen and Kimball, 1985, 1987). Seedlings emerging in late summer and early Autumn therefore likely avoid contact with hexazinone. Although the response of red sorrel to hexazinone is variable (Kennedy et al., 2011; Li, 2013), late emerging seedlings will likely avoid contact with other soil applied herbicides evaluated for weed control in lowbush blueberry. Future work should focus on determining the overwinter survival of seedlings emerging late in the season and the role of these seedlings in maintaining genetic diversity in established red sorrel populations.

3.7. Conclusions

The demography of red sorrel ramets and seedlings were studied in blueberry and bare soil patches in three lowbush blueberry fields in Nova Scotia between 2009 and 2011. Results of this study are summarized in a life-cycle model developed for red sorrel in lowbush blueberry. Three distinct components of red sorrel populations were identified in lowbush blueberry fields; the initial overwintering ramet population, the population of new ramets emerging within a given
season, and seedlings. The majority of flowering ramets (>70%) at each site were from the overwintering ramet cohort, with the remainder of the flowering ramet population comprised of ramets emerging in May and June. New ramets emerged throughout the season but the majority of these ramets (>90%) remained vegetative in the year of emergence. Ramet populations at each site were regulated by a cycle of ramet birth and death. A large net gain to ramet populations in the non-bearing year occurred, primarily due to lower ramet mortality in blueberry patches than in bare soil patches. Mortality was similar in both blueberry and bare soil patches in the bearing year and ramet populations tended to stabilize or decline during this phase of the production cycle. Final net ramet populations at the end of each season were comprised of the surviving flowering and vegetative ramets from both the initial overwintering and new ramet populations. Seedling survival averaged 19±4% across sites and indicates that new seedlings are regularly recruited into established ramet populations at the end of each season. The proposed life-cycle model provides the most accurate estimate to date of the proportional transition of red sorrel ramets and seedlings under field conditions in lowbush blueberry. This provides a benchmark for estimating the effect of management practices on the recruitment of new ramets and seedlings, as well as the ability to estimate the effects of these practices on the proportional composition of overwintering and new ramet populations.
Chapter 4: Temperature thresholds and degree-day models for red sorrel (*Rumex acetosella* L.) ramet sprouting, emergence, and flowering in lowbush blueberry (*Vaccinium angustifolium* Ait.) fields in Nova Scotia, Canada.

4.1. Abstract

*Rumex acetosella* L. is a common herbaceous creeping perennial weed in lowbush blueberry fields in Nova Scotia that spreads by seeds and an extensive creeping root system. Experiments were established to determine temperature thresholds for ramet sprouting from creeping root fragments and to develop growing degree-day (GDD) models for predicting ramet emergence and flowering under field conditions in lowbush blueberry fields in Nova Scotia. Ramets sprouted from root fragments of *R. acetosella* at temperatures as low as 1°C, with an optimum temperature for ramet sprouting at 21.6°C. Ramet sprouting was completely inhibited at temperatures above 35°C. Cumulative ramet emergence and flowering under field conditions were adequately explained as functions of GDD by a three-parameter power equation ($R^2=0.98$) and a four parameter logistic equation ($R^2=0.87$), respectively. Ramet emergence began between 110 and 265 GDD and continued throughout the season at each site. Model prediction for the initiation of emergence was 92 GDD, and 10, 50, 90, and 95% emergence were predicted to occur at 279, 1322, 2536, and 2696 GDD, respectively. Red sorrel ramets began to flower in the field between 308 and 515 GDD. Model prediction for the initiation of flowering was 289 GDD, and 10, 50, 90, and 95% flowering were predicted to occur at 376, 545, 877 and 1336 GDD, respectively. Model validation was conducted using two additional independent data sets for emergence and flowering and generally indicated good performance of the proposed models ($R^2$ and root-mean-square error values ranging from 0.96 to 0.99 and 4.0 to 13.8, respectively).
4.2. Introduction

Native stands of lowbush blueberry (*Vaccinium angustifolium* Ait.) are comprised of multiple genetically distinct clones that spread by rhizomes (Glass and Percival, 2000). These stands are managed on a two-year cycle in which fields are pruned in the first year (non-bearing year) and harvested in the second year (bearing year). Fields are managed to encourage the vegetative spread of blueberry clones into bare areas, but this also encourages the growth and spread of perennial weeds (Hall, 1959; McCully et al., 1991; Yarborough and Bhowmik, 1989).

Red sorrel (*Rumex acetosella* L.) is a common herbaceous perennial weed species in commercially managed lowbush blueberry (*Vaccinium angustifolium* Ait.) fields. Frequency of this species increased by 43% between the early 1980’s and the early 2000’s, and the plant is now established in over 90% of the lowbush blueberry acreage in Nova Scotia (Jensen and Sampson, unpubl. data; McCully et al., 1991). The lack of tillage and maintenance of low-pH soils associated with commercial lowbush blueberry production likely contribute to the persistence of red sorrel. Movement of red sorrel seed on blueberry harvesters is likely a frequent occurrence (Boyd and White, 2009), and control from the predominant herbicides used in lowbush blueberry is inconsistent (Kennedy et al., 2010; Kennedy et al., 2011; Li, 2013).

Red sorrel is dioecious and spreads by seeds and a shallow creeping root system (Kennedy, 2009; Sampson et al., 1990). Seedlings contribute to established red sorrel populations in lowbush blueberry fields (Chapter 3), but vegetative reproduction of ramets from the creeping root system is the primary means of population maintenance (Kennedy, 2009;
Sprouting of vegetative propagules such as creeping roots can be affected by factors such as propagule moisture content (Boose and Holt, 1999), propagule size and burial depth (Anbari et al., 2010; Edwards and Oliver, 2004; Ivany, 1997; Sciegienka et al., 2011), dormancy status of vegetative buds (Horvath et al., 2003; Klimešová and Klimeš, 2006), and temperature (Chachalis and Reddy, 2005; Holt and Orcutt, 1996). The majority of the red sorrel creeping root system in lowbush blueberry fields occurs in the upper 7 cm of soil (Chapter 3), and roots are not fragmented or buried by tillage in this production system. Lack of tillage and disturbance of the root system also limits the exposure of the root system to desiccation at the soil surface. Temperature alone has proven sufficient for predicting sprouting and emergence of vegetative propagules of other perennial weeds under similar field conditions (Donald, 2000; McAllister and Haderlie, 1985; Satorre et al., 1985; Webster and Cardina, 1999), but has not been evaluated for red sorrel in lowbush blueberry fields. Red sorrel ramet emergence in lowbush blueberry fields is season-long in Nova Scotia (Chapter 3), indicating that creeping roots of this species sprout under a wide range of temperatures. Basic physiological data regarding the temperature response of red sorrel creeping roots, however, is lacking (Stopps et al., 2011).

Ramets that emerge from the creeping root system of red sorrel are also developmentally segregated with established populations containing both vegetative and flowering ramets (Fujitaka and Sakai, 2007; Putwain and Harper, 1972). Flowering occurs primarily in overwintering or early-emerging ramets in lowbush blueberry fields (Chapter 3) and generally occurs during blueberry bloom (Hughes, 2012). Predicting weed emergence and phenological development is useful for relating development of weeds to that of the crop (Ghersa and Holt,
Predictive models for the emergence and development of lowbush blueberry ramets to tip dieback and flowering in Nova Scotia have recently been developed (White et al., 2012; Chapter 2) and now allow for this type of comparison. Emergence and flowering of the perennial weed spreading dogbane (*Apocynum androsaemifolium* L.) have recently been modeled as functions of GDD (Wu, 2010) in lowbush blueberry fields. Similar models for other important weed species such as red sorrel, however, are lacking.

The objectives of this research were to 1) determine the sprouting response of red sorrel creeping roots maintained at constant temperatures, 2) develop degree-day models to predict red sorrel ramet emergence and flowering in lowbush blueberry fields in Nova Scotia, and 3) to validate the proposed degree-day models with independent data sets collected in Nova Scotia.

### 4.3. Materials and Methods

#### 4.3.1. Root Material for Root Sprouting Experiments

Red sorrel roots were collected as needed from a wild blueberry field in Collingwood, Nova Scotia in September, October and November, 2010. Roots were collected from the top 5-10cm of soil using a garden rake, placed in paper bags and stored in a cooler until arrival at the lab where roots were placed in a 4°C cold room until needed. Roots were gently washed of excess soil under running water at the time of use, and no roots used in experiments were stored for more than three weeks.
4.3.2. Temperature Experiments

Three experiments were conducted to evaluate the effect of temperature on ramet sprouting from creeping roots. In all experiments, five 2 cm root fragments were placed in Petri dishes lined with two pieces of Whatman No. 1 9 cm diameter filter paper (Whatman Ltd., GE Healthcare Companies). Filter paper was moistened with 5 ml of distilled water just prior to placing roots in each dish. Petri dishes were then sealed with Parafilm™ and covered with aluminum foil to exclude light. Light was excluded in all experiments because light levels could not be kept constant in all incubators. A sprouted, upward pointing shoot on a root fragment was counted as a ramet when the shoot was at or exceeded 5 mm in length. In each experiment the total number of ramets per root fragment were counted in each petri dish five weeks after initiation of the experiment and expressed as the mean number of ramets per 2 cm root fragment. Each experiment was repeated once.

The objective of the first experiment was to determine the sprouting response of red sorrel root fragments grown under constant temperatures of 1, 2, 3, 4, and 5°C. Root fragments were placed in Precision Low Temperature incubators (GCA Corporation, Chicago, Illinois, USA) for temperatures of 1, 2, and 3°C, in a Conviron CMP5090 Controlled Environment Chamber (Conviron Controlled Environments Limited, Winnipeg, Manitoba, Canada) for the 4°C temperature, and a cold storage room for the 5°C temperature. Each treatment was replicated twelve times and results from both experimental runs were combined for analysis.

The objective of the second experiment was to determine the sprouting response of red sorrel root fragments grown under constant temperatures of 5, 10, 15, 20, 25 and 35°C. The 5°C
treatment was conducted in a cold storage room and the 10, 15, 20, and 25°C treatments were conducted in the same Precision Low Temperature Incubators used for the minimum temperature study. The 35°C treatment was conducted in a Thermo Scientific Lab Line General Purpose Incubator (Thermo Scientific, Dubuque, Iowa, USA). Each treatment was replicated eight times and results from both experimental runs were combined for analysis.

The objective of the third experiment was to determine the sprouting response of red sorrel root fragments grown under constant temperatures of 25, 30, 35, and 40°C. The 25°C treatment was conducted in the same incubator as experiment 2. The 30 and 35°C treatments were conducted in Thermo Scientific Lab Line General Purpose Incubators (Thermo Scientific, Dubuque, Iowa, USA), and the 40°C treatment was conducted in a CSE High Temperature Incubator (Chicago Surgical and Electrical Co., Melrose Park, Illinois, USA). Petri dishes in this experiment were monitored every 3-5 days for moisture, and 3-5ml of distilled water was added to each dish as needed. Each treatment was replicated eight times and results from both experimental runs were combined for analysis.

The mean number of ramets per 2cm root fragment in experiment one were analyzed using linear regression (PROC REG, SAS system for Windows Version 9.2, SAS Institute, Cary, NC). In experiment 2 a non-linear, 4-parameter Gaussian model was fitted to the mean number of ramets per 2cm root fragment. The model was of the form

\[
y = y_0 + a \exp(-0.5(\frac{x-x_0}{b})^2)
\]

where \(y\) is the mean number of ramets per 2cm root fragment, \(y_0\) is the value of \(x\) when \(y = 0\), \(x\) is temperature, \(x_0\) is the temperature that produces the peak mean number of ramets per 2cm root
fragment, $a$ is the theoretical maximum mean number of ramets per 2cm root fragment, and $b$ is a shape parameter. The model was fit using the Gauss-Newton algorithm in PROC NLIN of the SAS system for Windows Version 9.2 (SAS Institute, Cary, NC). Assessment of model fit was determined by calculating the coefficient of determination ($R^2$) and adjusted coefficient of determination ($R^2_{Adj}$), described below. Data for experiment 3 were subject to ANOVA (PROC GLM, SAS Institute, Cary, NC) with temperature modeled as a fixed effect.

4.3.3. Growing Degree-Day Models to Predict Ramet Emergence and Flowering Under Field Conditions

Data on red sorrel ramet emergence and flowering was collected during both the non-bearing and bearing years at three lowbush blueberry fields in Nova Scotia between 2009 and 2011 (Table 4.1). A total of eight quadrats were established for monitoring ramet emergence and flowering at each site; four in red sorrel patches occurring within blueberry clones and four in red sorrel patches occurring in bare soil areas between blueberry clones. Quadrat size was 0.09m$^2$, and all quadrat locations established in the non-bearing year were retained for bearing-year data collection at each site.

Emergence and flowering counts were initiated as early as possible in the spring of both the non-bearing and bearing years, generally by late April or early May. Flowering counts were conducted once or twice weekly at each site throughout spring and summer until no new flowering ramets were observed. All flowering ramets at each count were examined with a hand lens to identify male and female flower organs (stamens and pistils), and the total number of
Table 4.1. Description of study sites used to collect data for calibration and validation of growing degree-day models developed for red sorrel ramet emergence and flowering in lowbush blueberry fields in Nova Scotia, Canada. Non-bearing year sites established in 2009 and 2010 were retained for bearing year data collection in 2010 and 2011, respectively.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Production Year</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (jm)</th>
<th>Soil Type(^z)</th>
<th>Soil pH(^y)</th>
<th>Soil %OM(^y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purdy-2009</td>
<td>Non-bearing</td>
<td>45°35’34.904” N</td>
<td>63°50’49.932” W</td>
<td>114</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Purdy-2010</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wyvern-2009</td>
<td>Non-bearing</td>
<td>45°32’57.042” N</td>
<td>63°55’56.311” W</td>
<td>238</td>
<td>Sandy loam</td>
<td>4.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Wyvern-2010</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pigeon Hill-2010</td>
<td>Non-bearing</td>
<td>45°35’10.900” N</td>
<td>63°51’37.525” W</td>
<td>190</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Pigeon Hill-2011</td>
<td>Bearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


\(^y\) pH and % OM (% organic matter) determined from 4 soil cores taken to a depth of 10 cm at each site. Cores were combined to form a composite sample for each site. Composite samples submitted to the Nova Scotia Department of Agriculture Provincial Analytical Laboratory for analysis.
identifiable male and female flowering ramets were counted and marked with colored paper clips to keep flowering ramets separate over counting dates. Emergence counts were initiated at the same time as flowering counts but were conducted once or twice weekly throughout spring, summer, and fall until no new ramets emerged. Newly emerged ramets at each count were marked with colored elastics to keep emergence cohorts separate. Flowering and emergence counts were expressed on a percent cumulative scale for modeling purposes.

Hourly air temperature at each site was monitored using temperature loggers (HOBO Pro V2, Onset Computer Corporation, Cape Cod, Massachusetts). Data loggers were attached to wooden stakes and were located about 0.5m above the soil surface. Regional air temperature data from the nearest Environment Canada weather station were used to supplement field-based temperature data so that growing degree days (GDD) could be calculated starting on April 1 (day of year 91). Cumulative GDD’s were calculated using the formula:

\[
GDD = \sum_{i=1}^{n} (T_{\text{mean}} - T_{\text{base}})
\]

where \(T_{\text{mean}}\) is the mean daily air temperature, \(T_{\text{base}}\) is the lowest air temperature at which it is assumed ramet flowering or emergence will not occur, and \(n\) is the number of days over which GDD’s are calculated. In this equation, \(GDD = 0\) if \(T_{\text{mean}} \leq T_{\text{base}}\), similar to the approach used by Gordon and Bootsma (1993) for determining annual GDD accumulations in Atlantic Canada. Rainfall data for each site were obtained from the nearest Environment Canada weather station. Mean daily air temperature and rainfall data for each site is provided (Fig. 4.1).

Cumulative ramet flowering and emergence were plotted as functions of GDD. Fitting of non-linear equations, as well as parameter estimates for these equations, was conducted using the
Fig. 4.1. Daily mean air temperature (line) and rainfall (bars) during red sorrel ramet emergence and flowering at A) Purdy-2009, (B) Wyvern-2009, (C) Pigeon Hill-2010, (D) Purdy-2010, (E) Wyvern-2010, and (F) Pigeon Hill-2011. Mean daily air temperature was obtained from HOBO temperature loggers placed 0.5m above the soil surface at each site. Rainfall data for all sites were obtained from the Environment Canada weather station located at Nappan, Nova Scotia (45°45′34.400″ N, 64°14′29.200″ W, elevation 19.80m). Flowering data was collected between day of year 91 and 240. Emergence data was collected between day of year 91 and 334.
Gauss-Newton algorithm in PROC NLIN of the SAS system for Windows Version 9.2 (SAS Institute, Cary, NC). Percent cumulative flowering ramets (Y) was related to cumulative GDD with a four parameter logistic equation of the form:

\[
y = \frac{a+b}{1+(\frac{x}{x0})^c}
\]  

where \( y \) is percent cumulative flowering at any given GDD, \( a \) and \( c \) are shape parameters, \( b \) is the theoretical maximum percent cumulative ramet flowering, \( x \) is time in GDD, and \( x0 \) is the time, in GDD, until 50\% flowering. The base air temperature for ramet flowering was determined by iterating a series of base temperatures (0 to 10\(^\circ\)C in 1\(^\circ\)C intervals) in equation 2 until the best fit was obtained between percent cumulative ramet emergence and cumulative growing degree days (Izquierdo et al., 2009). The best fit was obtained for \( T_{\text{base}} \) equal to 0\(^\circ\)C. Given no current biological justification for using an alternative \( T_{\text{base}} \), 0\(^\circ\)C was chosen based on best fit and simplicity in data calculation in both the current study and for potential end-users of the proposed model.

Percent cumulative ramet emergence (Y) was related to cumulative GDD with a three parameter power equation of the form

\[
y = a + bx^c
\]  

where \( y \) is percent cumulative ramet emergence at any given GDD, \( a \) is the approximate value of \( y \) when \( x=0 \), \( x \) is time, in GDD, and \( b \) and \( c \) are shape parameters. The base air temperature for ramet emergence was determined through iteration, as described above. The best fit was obtained for \( T_{\text{base}} \) equal to 0\(^\circ\)C. Given no current biological justification for using an alternative \( T_{\text{base}} \), 0\(^\circ\)C
was chosen based on best fit and simplicity in data calculation in both the current study and for potential end-users of the proposed model.

Goodness of fit for the proposed models was determined by calculating the coefficient of determination ($R^2$) and adjusted coefficient of determination ($R^2_{Adj}$):

$$R^2 = 1 - \frac{\sum (y_{obs} - y_{pred})^2}{\sum (y_{obs})^2}$$  \hspace{1cm} [5]

and

$$R^2_{Adj} = 1 - \frac{n(1 - R^2)}{n - p}$$  \hspace{1cm} [6]

where $y_{obs}$ and $y_{pred}$ are the observed and predicted values, respectively, $n$ is the number of observations and $p$ is the number of parameters in the regression equation (Bowley, 2008), and the root-mean-square-error (RMSE):

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_{obs} - y_{pred})^2}$$  \hspace{1cm} [7]

Goodness of model fit was based on low RMSE and $R^2_{Adj}$ values close to 1. The proposed flowering and emergence models were validated with two additional site-years of flowering and emergence data that were not included in the model calibration. Flowering and emergence data from each site were expressed on a percent cumulative scale and plotted against cumulative GDD. Flowering and emergence predictions were calculated with the model and
plotted against observed flowering and emergence at each site, and the $R^2_{Adj}$ and RMSE described above were used to assess agreement between observed data and model predictions.

4.4. Results

4.4.1. Temperature Experiments

The mean number of ramets per 2cm root fragment increased linearly between 1 and 5°C (Fig. 4.2). Results of the linear regression indicate an increase of 0.185 ramets per 2cm root fragment between 1 and 5°C and a base temperature for ramet sprouting of -0.065°C (Fig. 4.2). The mean number of ramets per 2cm root fragment increased in a sigmoidal fashion up to temperatures near 20°C but then declined as temperatures approached 35°C (Fig. 4.3). The Gaussian model fit the data well and predicted a maximum mean number of ramets per 2cm root fragment at a temperature of 21.6°C (Table 4.2). Mean ramet data for experiment 3 could not be made to conform to the assumptions for the variance analysis, but no ramets sprouted at temperatures greater than 35°C (Table 4.3).

Table 4.2. Parameter estimates and goodness of fit statistics for the Gaussian equation fit to red sorrel ramet sprouting at constant temperatures of 5, 10, 15, 20, 25, and 35°C.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Parameters$^2$</th>
<th>( a )</th>
<th>( b )</th>
<th>( y0 )</th>
<th>( x0 )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( y = y0 + a \cdot \exp(-0.5(x-x0)^2/b) )</td>
<td></td>
<td>1.1657</td>
<td>9.9013</td>
<td>-0.2672</td>
<td>21.6647</td>
<td>.9177</td>
</tr>
</tbody>
</table>

$^2$Gaussian model parameters; \( a \) and \( b \) are shape parameters; \( y0 \) is the value of \( x \) when \( y = 0 \); \( x0 \) is the temperature that produces the peak mean ramet number.
Table 4.3. Effect of constant temperatures on the mean number of red sorrel ramets per 2cm root fragment.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mean Ramets 2cm root fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.94 ± 0.126</td>
</tr>
<tr>
<td>30</td>
<td>0.94 ± 0.134</td>
</tr>
<tr>
<td>35</td>
<td>0.04 ± 0.027</td>
</tr>
<tr>
<td>40</td>
<td>0.0 0.0</td>
</tr>
</tbody>
</table>

Fig. 4.2. The relationship between the mean number of ramets per 2cm red sorrel root fragment and constant temperatures of 1, 2, 3, 4, and 5 °C. Symbols are the mean number of ramets per 2cm root fragment. Error bars represent one SE of the mean. The line is a fitted linear regression equation.
Fig. 4.3. The relationship between the mean number of ramets per 2cm red sorrel root fragment and constant temperatures of 5, 10, 15, 20, 25, and 35°C. Symbols are the mean number of ramets per 2cm root fragment. The line is a fitted non-linear Gaussian equation of the form $y = y_0 + a \exp(-0.5\left(\frac{x-x_0}{b}\right)^2)$. Parameter estimates and goodness of fit statistics for the Gaussian model are provided in Table 4.2.
4.4.2. Growing Degree-Day Models to Predict Ramet Emergence and Flowering Under Field Conditions

Red sorrel ramet emergence began between 110 and 265 GDD (Fig. 4.4A). Emergence continued throughout the season at each site and ramet populations reached 90% emergence between 2091 and 2565 GDD (Fig. 4.4A). The proposed power model fit the field data well and accurately predicted emergence in the field as a function of GDD (Fig. 4.4A, Table 4.4). Model prediction for the initiation of emergence was 92 GDD, and 10, 50, 90, and 95% emergence were predicted to occur at 279, 1322, 2536, and 2696 GDD, respectively. Red sorrel ramets began to flower in the field between 308 and 515 GDD (Fig. 4.4B). Flowering generally occurred quite rapidly at each site and ramet populations reached 90% flowering between 623 and 1308 GDD (Fig. 4.4B). The proposed model fit the field data well and accurately predicted flowering in the field as a function of GDD (Fig. 4.4B, Table 4.4). Model prediction for the initiation of flowering was 289 GDD, and 10, 50, 90, and 95% flowering were predicted to occur at 376, 545, 877 and 1336 GDD, respectively.

![Fig. 4.4. Calibration of GDD (T_{base}=0°C) models for predicting A) emergence and B) flowering of red sorrel ramets in lowbush blueberry fields in Nova Scotia, Canada.](image-url)
Table 4.4. Parameter estimates and goodness of fit statistics for calibration of the power and logistic equations fit to red sorrel ramet emergence and ramet flowering, respectively, as a function of GDD (T_{\text{base}}=0^\circ\text{C}).

<table>
<thead>
<tr>
<th>Model</th>
<th>Site-year</th>
<th>Equation</th>
<th>Model Parameters$^2$</th>
<th></th>
<th></th>
<th></th>
<th>R$^2$</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergence</td>
<td>Purdy-2009</td>
<td>$y = a + bx^c$</td>
<td>$a$</td>
<td>$b$</td>
<td>$c$</td>
<td>$x_0$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-4.7767</td>
<td>0.1296</td>
<td>0.8413</td>
<td>-</td>
<td>0.98</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.7556)</td>
<td>(0.0546)</td>
<td>(0.0506)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wyvern-2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pigeon Hill-2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pigeon Hill-2011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowering</td>
<td>Purdy-2009</td>
<td>$y = \frac{a + b}{1 + (\frac{x}{x_0})^c}$</td>
<td>$a$</td>
<td>$b$</td>
<td>$c$</td>
<td>$x_0$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-1.9855</td>
<td>97.5330</td>
<td>-5.6300</td>
<td>532.3</td>
<td>0.87</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4.2197)</td>
<td>(5.4659)</td>
<td>(0.9479)</td>
<td>(17.4672)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Purdy-2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wyvern-2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pigeon Hill-2011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^2$Emergence model parameters, $a$=approximate value of $y$ when $x=0$, $b$ and $c$=shape parameters; Flowering model parameters, $a$=shape parameter, $b$= theoretical maximum, $c$=shape parameter, $x_0$= time, in GDD, until 50% flowering.
Model predictions for red sorrel ramet emergence and flowering generally agreed with observed values (Fig. 4.5), indicating good general performance of these models for predicting emergence and flowering under field conditions. The primary exception was the deviation of the observed ramet emergence from the predicted emergence at Purdy-2010 (Fig. 4.5A). This was also reflected by the lower $R^2_{Adj}$ and higher RMSE associated with the observed and predicted emergence at this site (Table 4.5). All other models, however, fit the data well and had high $R^2_{Adj}$ and low RMSE (Table 4.5).

<table>
<thead>
<tr>
<th>Model Validated</th>
<th>Site-Year</th>
<th>$R^2_{Adj}$</th>
<th>RMSE $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergence</td>
<td>Purdy-2010</td>
<td>0.96</td>
<td>13.8</td>
</tr>
<tr>
<td></td>
<td>Wyvern-2009</td>
<td>0.99</td>
<td>4.0</td>
</tr>
<tr>
<td>Flowering</td>
<td>Wyvern-2010</td>
<td>0.99</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>Pigeon-Hill-2010</td>
<td>0.99</td>
<td>6.9</td>
</tr>
</tbody>
</table>

$^2$RMSE = Root mean square error

### 4.5. Discussion

Root fragments of *R. acetosella* produced ramets at temperatures as low as 1°C (Fig. 4.2), with an optimum temperature for ramet sprouting at approximately 21.6°C (Table 4.2). Ramet growth was completely inhibited at temperatures above 35°C (Table 4.3). This is the first report of estimated temperature thresholds for root sprouting in this species. It is unclear why the mean number of ramets per root fragment was higher at 5°C in the low temperature experiment than in the optimum temperature experiment (Figs. 4.2 and 4.3). Roots for the low temperature experiment were collected in late September as opposed to the mid-November collection date for
Fig. 4.5. Observed and model predicted red sorrel ramet emergence (A and B) and flowering (C and D) in relation to growing degree-days (GDD’s) calculated from air temperature ($T_{\text{base}}=0^\circ\text{C}$). Symbols are the mean of 8 observations. Lines are calibrated model predictions.
the roots used in the optimum temperature experiment. The number of shoots per creeping root fragment of some creeping perennial species is reported to decline in late autumn and winter when compared to warmer months (Liew et al., 2012; Swan and Chancellor, 1976). Shultz and Burnside (1979) indicated the importance of a similar cycle of root bud activity on timing of root collection for greenhouse experiments with hemp dogbane (Apocynum cannabinum). Future research of root sprouting activity of R. acetosella should be conducted with roots collected using more consistent sampling times across experiments.

Similar sprouting responses to temperature are observed in creeping roots of other perennial weeds, though differences in threshold and optimum temperatures for sprouting are common among studied species. Canada thistle (Cirsium arvense) is generally thought to produce root buds at temperatures near 0°C (Donald, 2000; McAllister and Haderlie, 1985), though root sprouting in some populations of this species is inhibited at temperatures near 5°C (Hamdoun, 1972). The optimum temperature for root sprouting of C. arvense is reported to be 15°C (Hamdoun, 1972), slightly lower than the optimum of 21.6°C found for root sprouting in R. acetosella. Most other perennial weeds with creeping roots studied occur as weeds in cropping systems in the southern United States and therefore have temperature thresholds representative of the predominant climates in which they inhabit. Creeping roots of honeyvine milkweed (Cynanchum laeve) collected in Oklahoma failed to sprout at temperatures below 15°C but had an optimum temperature for sprouting between 20 and 30°C (Soteres and Murray, 1982). Although similar optimum temperatures were found for C. laeve and R. acetosella, C. laeve required much warmer temperatures for the initiation of sprouting than R. acetosella. Similarly, rootstock sprouting of the perennial vines Brunnichia ovata and Campsis radicans in Mississippi was
inhibited or reduced at 15°C but exhibited optimal sprouting at temperatures between 30 and 40°C (Chachalis and Reddy, 2005). Temperature thresholds for vegetative propagules of other perennial weeds have primarily been estimated for rhizomatous or tuberous species such as *Sorghum halapense* (Holt and Orcutt, 1996; Hull, 1970) *Agropyron repens* (L.) Beauv. (Leaky et al., 1978) and *Cyperus spp.* (Holt and Orcutt, 1996; Nishimoto, 2001). Similar studies on common perennial species from more northern regions, including many perennial weeds in lowbush blueberry fields, are lacking. Therefore little information is available for comparison of temperature thresholds for species acclimated to a similar climactic regime as the red sorrel plants used in this study.

The growing degree-day models developed for red sorrel emergence and flowering will have utility in both management and future study of this species in lowbush blueberry fields. In previous work it was found that the majority of flowering ramets (>70%) are confined to an overwintering ramet cohort persisting from the previous season (Chapter 3). The actual proportion of the overwintering cohort that flowers, however, ranges from 30 – 70% and thus all overwintering ramets do not flower (Chapter 3). Flowering of overwintering plants under field conditions often depends upon size of established plants prior to the onset of winter (Gross, 1981; Klinkhamer et al., 1987a), a factor often affected by the time of seedling emergence during the growing season (Gross, 1980; Weaver and Cavers, 1979). Similar size differences have not been confirmed in field populations of red sorrel ramets. The availability of a degree-day model to predict proportional emergence of new ramets provides an efficient tool for monitoring and tracking new ramets within a given season and assessing the developmental fate of those ramets in subsequent seasons. The model should therefore prove useful for improving our understanding
of the flowering biology of red sorrel in lowbush blueberry fields in Nova Scotia. The deviation of the observed values from the predicted values at Purdy-2010 (Fig. 4.5A) indicate that additional validation data sets, however, would be useful for further evaluation of the predictive capability of the proposed model.

In terms of management, the degree-day model should improve our ability to predict the approximate timing of peak ramet populations in lowbush blueberry fields and therefore improve the timing of herbicide applications to manage this species. For example, autumn applications of paraquat following blueberry pruning have been found to significantly reduce both overwintering ramet populations and the density of flowering ramets in the subsequent non-bearing year (Chapter 5). Blueberry growers generally prefer to prune harvested fields in late autumn due to improved cutting efficiency of blueberry stems with flail mowers following the onset of cold weather. Coordinating blueberry pruning with the onset of peak ramet populations through the use of the proposed degree-day model should provide a basis for maximizing the delay in autumn pruning without compromising weed control.

The general emergence pattern of red sorrel ramets as a function of GDD is much more prolonged than is reported for emergence of other perennial weeds from creeping roots. For example, ramet emergence of spreading dogbane (*Apocynum androsaemifolium* L.) in lowbush blueberry fields was much more rapid, with ramet populations reaching 50 and 100% emergence at 184 and 420 GDD (*T*_base_=6°C), (Wu, 2010). Similar GDD thresholds are also reported for *Apocynum cannabinum* ramet emergence from creeping roots in Ohio (Webster and Cardina, 1999). Ramets of *C. arvense* reached 1 and 80% emergence from creeping roots at 197 and 587
GDD \( (T_{\text{base}}=0^\circ\text{C}) \) in spring wheat in North Dakota (Donald, 2000). Inherent differences in the biology of these species when compared to red sorrel, however, may make comparisons of emergence patterns difficult. Ramets of \textit{A. cannabinum} and \textit{C. arvense} dieback to soil level each year and do not generally persist for more than one season (Moore, 1975; Robison and Jeffrey, 1972). In contrast, surviving red sorrel ramets from a single season persist for at least two growing seasons. These species therefore exhibit inherent differences in the demographic aspects of ramet production at the genet level that ultimately affect ramet emergence patterns observed in the field.

The base temperature of 0\(^\circ\text{C}\) identified through the iterative process outlined above was in close agreement with the base temperature of -0.065\(^\circ\text{C}\) estimated from the low temperature threshold root sprouting experiment (Fig. 4.2). Temperatures below freezing were not directly tested in our experiments, so 0\(^\circ\text{C}\) was used as an initial estimate until additional research can be conducted. Base temperatures for seed germination or sprouting of vegetative propagules are often species specific (Holt and Orcutt, 1996; Steinmaus et al., 2000), though our estimate of 0\(^\circ\text{C}\) for red sorrel is in general agreement with that used for modeling emergence of \textit{C. arvense} from creeping roots in North Dakota (Donald, 2000) and dandelion from rootstock in Western Canada (Hacault and Van Acker, 2006). A higher base temperature has been reported for modeling the emergence of \textit{A. cannabinum} and \textit{A. androsaemifolium} from creeping roots (Webster and Cardina, 1999; Wu, 2010). Base temperatures for emergence of ramets from creeping roots of other perennial species, however, are lacking. The low base temperature for root sprouting is likely an adaptation to the climate of the study region and allows \textit{R. acetosella} to produce ramets and maintain genet growth throughout the entire growing season. In terms of an upper
temperature threshold, ramet sprouting was only inhibited at temperatures above 35°C (Table 4.3). This temperature is well above the mean maximum temperatures observed at the study sites (Fig. 4.1) and indicates that models incorporating impacts of high temperature thresholds on emergence are likely unnecessary.

The growing degree-day model developed to predict red sorrel ramet flowering is a useful tool for comparison of GDD thresholds for both blueberry and red sorrel ramet flowering under field conditions. Predicted GDD thresholds for red sorrel flowering were similar to thresholds previously established for lowbush blueberry (White et al., 2012; Chapter 2), with 50% of blueberry and red sorrel ramets having open flowers at 477 and 545 GDD ($T_{\text{base}}=0^\circ$C), respectively. Peak blueberry bloom occurs between 562 and 565 GDD (White et al., 2012; Chapter 2), and so red sorrel flowering occurs during a critical time for pollination of the lowbush blueberry. Red sorrel flowers are foraged by introduced pollinators (Hughes, 2012), though the overall impact of this foraging on pollination of the lowbush blueberry is unclear. Hughes (2012) also found large amounts of pollen from male red sorrel flowers deposited inside open blueberry flowers. Red sorrel pollen increased the incidence of *Botrytis cinerea* infection on blueberry flowers under controlled conditions (Hughes, 2012), though the impact of red sorrel pollen on disease incidence under field conditions has not been confirmed. Lowbush blueberry growers nonetheless report increased requirement for fungicide applications to control outbreaks of *Botrytis cinerea* in fields with heavy red sorrel infestations, a requirement potentially accentuated by the overlapping flowering of the two species.
The ability to accurately compare the flowering time of weeds to that of the lowbush blueberry across seasons may also have impacts on the ability of growers to manage populations of native pollinators. Native bees that pollinate lowbush blueberry benefit from the presence of other plant species that flower before and after the lowbush blueberry (Argall et al., 1998; MacKenzie et al., 2004, Stubbs et al., 1992). The development of degree-day models to predict flowering of red sorrel and other weed species in lowbush blueberry fields could therefore be used as a classification system to help determine the potential benefits of some weed species for maintaining native pollinator populations. Stubbs et al. (1992) provided a list of nearly fifty species of alternative forage plants for native bees in Maine, as well as the approximate calendar dates for flowering of these species. A similar approach could be taken in Nova Scotia using degree-days rather than calendar date to relate flowering time of alternative forage plants to that of the lowbush blueberry. Degree-day thresholds for flowering of *A. androsaemifolium* have been reported in lowbush blueberry fields in Nova Scotia (Wu, 2010), and similar data are being compiled for *Hieracium* spp. on Prince Edward Island (Eriavbe, personal communication). Ongoing collection of similar data should contribute to the development of a comprehensive list of flowering times for important weed species in lowbush blueberry fields.

Weed species flowering during blueberry bloom may also provide benefits to both native and introduced pollinators. For example, Hughes (2012) has indicated that pollen from red sorrel flowers might be an important food source for introduced pollinators such as honeybees, particularly if pollen stores of introduced hives are low. Although the quality of red sorrel pollen as a food source for either native or introduced pollinators is unknown, small amounts of pollen from *Rumex* spp. have been detected in the pollen loads of native pollinators in Maine (Stubbs et
al., 1992). Although purely speculation, moderate to low infestations of flowering red sorrel ramets may be beneficial to the health of native and introduced pollinators in lowbush blueberry fields.

Finally, the proposed model for predicting flowering under field conditions provides a basis for comparison of the flowering response of red sorrel in controlled experiments. Recent work has begun to confirm the vernalization and photoperiod requirements for ramet flowering in this species (Chapter 5). The length of time required for flowering following various vernalization and photoperiod treatments in controlled experiments is an important measure of the success of these treatments (Chouard, 1960; Clough et al., 2001). The availability of a GDD model for flowering in field populations therefore provides a valuable reference for assessing the time required for flowering following experimental treatments under controlled conditions.

4.6. Conclusions

Red sorrel ramets sprouted from creeping root fragments at temperatures as low as 1°C. The number of ramets per creeping root fragment increased linearly between 1 and 5°C with an increase of 0.185 ramets per root fragment with each 1°C change in temperature over this range. The intercept of the linear regression indicated a base temperature for ramet sprouting of -0.065°C. The optimum temperature for ramet sprouting was 21.6°C and no ramets sprouted at temperatures above 35°C. Growing degree-day models were developed to predict ramet emergence and flowering under field conditions in lowbush blueberry fields in Nova Scotia. Ramets were predicted to emerge at 92 GDD and reach 10, 50, 90, and 95% emergence at 279, 1322, 2536, and 2696 GDD, respectively. Ramets were predicted to flower at 289 GDD and
reach 10, 50, 90, and 95% flowering at 376, 545, 877 and 1336 GDD, respectively. Models were validated using two additional independent data sets for emergence and flowering and validation generally indicated good performance of the proposed models.

5.1. Abstract

Studies were initiated to examine the role of photoperiod, vernalization, pre-vernalization stimulus, and pre and post-vernalization ramet removal on flowering of red sorrel (*Rumex acetosella* L.) ramets and seed plants in Nova Scotia, Canada. Red sorrel ramets established from creeping roots collected from established field populations had an obligate vernalization requirement for flowering and less than 1% of plants established from seed collected from established field populations flowered without vernalization. Ramets and seed plants maintained under constant 16 hour, 14 hour, or 8 hour photoperiods did not flower. Ramets established from creeping roots and maintained under pre and post-vernalization photoperiods of 16 hours flowered following 12 weeks of vernalization at 4.5 ± 0.1°C. In contrast, ramets transferred to an 8 hour photoperiod following 12 weeks of vernalization at 4.5 ± 0.1°C remained vegetative. Ramets therefore require vernalization followed by long days for flower induction. Ramets maintained under an 8 hour photoperiod for 12 weeks and transferred to 16 hour photoperiods without vernalization did not flower. Ramets grown under an 8 hour photoperiod for 8 weeks prior to transfer to a 14 hour photoperiod also remained vegetative. Ramets therefore did not flower in response to increasing photoperiod. Ramets established from creeping roots and vernalized for durations of up to 10 weeks at 6°C did not flower. In contrast, about 10, 65, and 65% of seed plants flowered following 5, 10 and 15 weeks of vernalization, respectively, at 4.5 ± 0.1°C. Pre and post-vernalization ramet removal significantly reduced the density of flowering ramets in both field and controlled conditions. Exposure of ramets established from creeping
roots to decreasing photoperiod prior to vernalization for 16 weeks at 6°C significantly increased flower frequency over ramets maintained under constant photoperiod prior to vernalization. Exposure of ramets to decreasing temperature prior to vernalization had no effect on flowering frequency. In general, vernalization at temperatures near 4°C provided a more consistent flowering response than vernalization at 6°C. Therefore, temperatures near 4°C are recommended for future studies examining the effects of vernalization on the flowering biology of red sorrel.
5.2. Introduction

Maintaining a balance between vegetative and flowering meristems is critical to the life-history of polycarpic herbaceous perennials (Amasino, 2009; Battey, 2000) and contributes to the persistence of some of these plants as weeds (Leaky, 1981). A balance between vegetative and flowering meristems is maintained in herbaceous creeping perennials through the production of dormant or non-dormant buds on vegetative reproductive structures (Hartnett and Bazzaz, 1985b; McAllister and Haderlie, 1985; Werner et al., 1980), or through the production of vegetative and flowering ramets (Araki and Ohara, 2008; Noble et al., 1979; Worthen and Stiles, 1986).

Red sorrel (Rumex acetosella L.) is a common ramet-producing herbaceous creeping perennial species in commercially managed lowbush blueberry (Vaccinium angustifolium Ait.) fields and is now established in over 90% of the acreage of this crop in Nova Scotia (Jensen and Sampson, unpubl. data; McCully et al., 1991). The plant is dioecious and spreads by seeds and a shallow creeping root system (Kennedy, 2009). Seedlings contribute to established populations (Chapter 3), but these populations tend to be maintained predominantly by vegetative reproduction of ramets from the creeping root system (Kennedy, 2009; Putwain et al., 1968; Putwain and Harper, 1970). Red sorrel ramet populations are comprised of both vegetative and flowering ramets (Fujitaka and Sakai, 2007; Putwain and Harper, 1972), though the factors affecting red sorrel ramet development are not well understood.

Developmental fate of ramets of herbaceous creeping perennials is thought to be controlled by both biotic and abiotic factors (Cook, 1985). Flowering in field populations of red
sorrel ramets can occur throughout the season (Escarré and Thompson, 1991), but tends to be observed most frequently in early to mid-summer in most temperate regions (Fujitaka and Sakai, 2007; Harris, 1970; Korpelainen, 1992). Red sorrel ramets tend to flower in lowbush blueberry fields around mid-June in Nova Scotia (Kennedy, 2009). This generally follows the early-season peak in ramet density reported by Kennedy et al. (2010) and may indicate that flowering is confined to early-emerging ramets. However, initial ramet counts at some study sites included what appeared to be overwintering ramets persisting from the previous season (Kennedy, 2009; Kennedy et al., 2010). In a recent demographic study of field populations of red sorrel ramets in lowbush blueberry (Chapter 3), the majority of flowering ramets (>70%) were found to be confined to an overwintering cohort of ramets persisting from one season to the next. These data indicate that abiotic factors, such as vernalization and photoperiod, likely play an important role in the regulation of flowering in field populations of red sorrel ramets in lowbush blueberry. The importance of these factors in regulating ramet development in herbaceous perennials has been stressed (Cook, 1985; Sachs, 2002), though empirical data to support this in creeping herbaceous perennials is limited.

Knowledge of the impact of abiotic factors on flowering in red sorrel is limited primarily to the impacts of photoperiod. The plant is reported to remain vegetative under short days (Listowski and Jackowska, 1964). Flowering will occur after 130 to 140 days under long days, but this duration is shortened to about 40 days when plants are grown under short days prior to transfer to long days (Bavrina et al., 1991). Flowering has also been reported to occur under long days and ambient greenhouse conditions (Lovett Doust and Lovett Doust, 1987; Zimmerman and Lechowicz, 1982). The large portion of flowering ramets found in the overwintering ramet
cohort in lowbush blueberry fields, however, indicates that factors other than photoperiod are involved in the regulation of flowering in red sorrel populations in Nova Scotia. The predominance of flowering in overwintering ramets has primarily been reported in some commonly studied perennial grass and sedge species. Vegetative ramets of the sedge species *Carex arenaria* emerging in a given year require winter vernalization to flower in the following year (Noble et al., 1979). Similar behavior is noted in perennial grasses such as *Deschampsia caespitosa* (Davy, 1982), *Festuca spp.* (Wycherly, 1954), *Danthonia caespitosa* (Hodgkinson and Quinn, 1978), and *Lolium perenne* L. (McCown and Peterson, 1964). However, ramets of very few herbaceous broadleaf creeping perennials have been documented to require vernalization to confer flowering competency. With the exception of *Leucanthemum vulgare* (Heide, 1995), *Stellaria longipes* (MacDonald et al., 1984) and *Solidago nemoralis* and *S. albopilosa* (Walck et al., 1999), ramets of few herbaceous broadleaf creeping perennials, including red sorrel, have been reported to require vernalization for flowering. Harris (1970) has indicated that low temperatures may be required for flowering in some red sorrel populations, though this was based primarily on observations that plants collected from certain regions required more than one year of growth to flower. Similar flowering responses have been reported under field conditions for ramets of *Solidago shortii* (Walck et al., 1999) and *Cryptotaenia Canadensis* (Baskin and Baskin, 1988); very few ramets flower in the year of emergence under field conditions whereas plants established in heated greenhouses readily flower soon after establishment. Critical size requirements (Baskin and Baskin, 1988) or exposure of ramets to unfavorable growing conditions during the first year of growth (Walck et al., 1999), rather than vernalization, were cited as causes for observed delays in flowering under field conditions. It is unknown whether vernalization or lack of adequate ramet growth in the year of emergence
restrict flowering to overwintering red sorrel ramets in lowbush blueberry fields in Nova Scotia as no studies on red sorrel flowering biology have been conducted in this region.

The vernalization response in some species is also affected by pre-vernalization stimuli such as decreasing photoperiod and temperature. For example, Foley et al. (2009) found that *Euphorbia esula* plants required exposure to decreasing temperatures prior to vernalization for flower induction to occur. Thus, exposure to appropriate environmental stimuli prior to vernalization can be as important as vernalization itself with regards to conferring flowering competency. The proportion of overwintering red sorrel ramets that flower in lowbush blueberry fields varies but ranges from 39-75% (Chapter 3). Therefore, exposure to cold treatment alone appears to be insufficient to induce flowering competency in some ramets. It has already been indicated that red sorrel responds to short days with an accelerated flowering response after transfer to long days (Bavrina et al., 1991). The flowering response of ramets may therefore respond to changes in temperature or daylength prior to the onset of vernalizing temperatures in late fall and early winter.

Finally, the fact that flowering ramet populations are comprised primarily of ramets from the overwintering cohort provides opportunities for the development of weed management strategies designed to prevent the establishment of overwintering ramet populations, and thus reduce the density of flowering ramets in subsequent years. Similar approaches have been used for the management of monocarpic perennials and have proven successful (Medd and Lovett, 1978). Control of red sorrel in lowbush blueberry currently relies heavily on preemergence applications of hexazinone in early spring or pronamide in late fall. Red sorrel response to
hexazinone, however, is variable and generally unacceptable in fields with a long history of hexazinone use (Kennedy, 2009, 2010; Li, 2013). Control associated with autumn applications of pronamide is the most reliable option (Hughes, 2012), but is plagued by the unpredictability of climatic requirements essential for successful weed control with this product. The establishment of a distinct overwintering ramet population, combined with the critical role of this population in sexual reproduction and seed production, may provide the opportunity to implement fairly simple strategies oriented towards removing the overwintering ramet population prior to exposure to the conditions that confer flowering competency. This could prevent flowering and seed production, thus restricting the long distance dispersal of established genets that is required for the establishment of new populations (Abrahamson, 1980).

This research study was conducted to test the hypothesis that red sorrel ramets in lowbush blueberry fields in Nova Scotia require vernalization to confer flowering competency. Specific objectives of the research were to 1) determine if red sorrel ramets have an absolute requirement for vernalization to flower, 2) determine the effects of vernalization and photoperiod on the induction of ramet flowering, 3) determine the effect of vernalization duration on the induction of ramet flowering, 4) determine the effect of pre and post-vernalization ramet removal on flowering ramet density under field and controlled conditions, and 5) determine if red sorrel ramets require exposure to pre-vernalization stimuli such as decreasing photoperiod or temperature prior to vernalization to induce flowering competency.
5.3. Materials and Methods

5.3.1. Source of Plant Material

Unless otherwise stated, all plants were established from creeping roots or seeds collected from established red sorrel populations in commercial lowbush blueberry fields in Nova Scotia. Creeping roots were collected from established red sorrel populations at Mt. Thom (45°29’30.373” N, 62°59’25.200” W) or Pigeon Hill (45°35’10.900” N, 63°51’37.525” W) and seeds were collected at Pigeon Hill. Roots were excavated from the upper 5-10cm of soil using a shovel and placed in a cooler immediately after collection. Roots were then brought back to the lab, gently washed under running tap water, and cut into segments roughly 5cm in length. Root fragments were then placed in plastic greenhouse trays lined with moist paper towels, covered with an additional layer of moist paper towel, and incubated in the dark at a constant temperature of 20°C. Roots were checked daily for sprouting, and sprouted root fragments were planted for each experiment within 7-10 days after placement of roots in the incubation chamber. Seeds were collected by hand from mature ramets in late summer, placed in paper envelopes and stored at room temperature until use. Seeds were germinated by placing them in sealed petri dishes lined with filter paper moistened with 5ml of 2% KaNO₃. Petri dishes were covered with aluminum foil to exclude light and were kept in the lab at room temperature. Seeds germinated within 5-7 days, and germinated seeds were planted as indicated for each experiment. No seeds were stored for more than 6 months prior to use.

5.3.2. Growing Conditions and General Plant Maintenance

Unless otherwise stated, all experiments were conducted in a greenhouse or on illuminated shelves in a growth facility. Photoperiod in the greenhouse consisted of natural
daylight extended to 14 hours with fluorescent bulbs providing an average photosynthetic photon flux density (PPFD) of 171 ± 8.8 μmol m⁻² s⁻¹ at pot level. Mean greenhouse temperature was 22.4 ± 0.06°C. Lighting in the growth facility was provided by fluorescent bulbs providing an average PPFD of 111.5 ± 3.2 μmol m⁻² s⁻¹ at pot level. The growth facility was maintained at a constant temperature of 24.4 ± 0.2°C. All plants were watered as needed. In experiments with plants grown in cell packs, all cell packs were re-randomized and fertilized with a 0.12% solution of 20-20-20 general fertilizer blend every 2 weeks at a rate of 100ml of fertilizer solution per cell pack. In the ramet removal experiment using plants established from seed, all pots were re-randomized and fertilized with a 0.12% solution of 20-20-20 (N-P-K) general fertilizer blend every 2 weeks at a rate of 200ml of fertilizer solution per pot starting when plants had reached the 2-4 true leaf stage. In the experiment using ramets established from soil cores, each pot was fertilized with a 0.12% solution of 20-20-20 general fertilizer blend every 2 weeks at a rate of 50ml of fertilizer solution per pot. Plants exposed to vernalization were not fertilized or re-randomized during the vernalization treatment in any experiments.

5.3.3. Plant Acclimation and Vernalization

All plants exposed to a vernalization treatment were subject to pre and post-vernalization acclimation in a 13 or 15°C germination cabinet for the specified duration outlined in each experiment. Acclimation was conducted under an 8-hour photoperiod with a mean photosynthetic photon flux density (PPFD) of 22.7 ± 1.8 μmol m⁻² s⁻¹ at pot level. Vernalization consisted of exposure of ramets to constant 6°C in a germination cabinet or a mean temperature of 4.5 ± 0.1°C in a cold storage facility. The method used is specified for each experiment. Photoperiod and PPFD in the germination cabinet was the same as indicated for the acclimation
chamber. The cold storage facility was maintained under an 8-hour photoperiod provided by fluorescent bulbs providing an average PPFD of $52.6 \pm 2 \, \mu$mol m$^{-2}$ s$^{-1}$ at pot level.

5.3.4. General Data Collection and Flowering Assessment

Each experiment was repeated once unless otherwise stated. In each cell pack or pot in each experiment the following data were recorded: 1) the pre and post vernalization ramet density and ramet leaf number or leaf number of plants grown from seed, 2) days after vernalization until the first ramet or plant grown from seed was observed to bolt, 3) density and leaf number of flowering ramets or leaf number of flowering plants grown from seed, 4) stem height of flowering ramets or plants grown from seed, 5) final density and leaf number of surviving vegetative and flowering ramets or surviving plants grown from seed at the end of each experiment, and 6) number of dead flowering ramets or survival of plants grown from seed. Additional data collection specific to a given experiment is outlined as required. Bolting and flowering were considered to have occurred in each cell pack or pot if at least one ramet or plant grown from seed bolted or flowered. Ramets and plants grown from seed were considered to have bolted when the elongated flowering stem could be observed. Ramets and plants grown from seed were considered to have flowered when the pistils or stamens of open flowers were visible to the naked eye. Each cell pack or pot was therefore scored for flowering based on a categorical basis (yes or no), and the frequency of cell packs or pots with flowering ramets or plants grown from seed was determined for each treatment in each experiment.
5.3.5. Experiment 1 - Effect of Vernalization and Photoperiod on Ramets Established from Soil Cores

The objectives of this experiment were to 1) determine the main and interactive effects of photoperiod and vernalization on the induction of flowering in red sorrel ramets, and 2) determine the suitability of bulk density soil cores for establishing red sorrel ramets for experimental purposes. Red sorrel ramets were established from soil cores collected from red sorrel patches in a wild blueberry field in Londonderry, Nova Scotia (45°26’21.280” N, 63°32’44.640” W). Cores were collected from several dense patches of male and female red sorrel clones using a soil bulk density core sampler with a core volume of 331cm³. The core sampler contained a main core and a small spacer ring at the top of the core. Soil and plant material in the spacer portion of the core was removed with a knife in the field, and the remaining soil was depressed into the core to leave an approximate 2cm gap at the top of the core. Cores were then placed in plastic boxes and brought back to the lab. Intact cores were then transferred to 10cm diameter (707cm³) plastic pots lined with coarse sand. Pots were then randomly selected for each treatment. Each pot was considered an experimental unit, and 7 replicate pots were used for each treatment. All pots were moved to their respective photoperiod at the time that treatments were assigned.

The experiment was designed using a 3 X 3 factorial treatment arrangement in a completely randomized design. Treatment factors were photoperiod (8hr, 14hr, or increasing) and vernalization period (0, 6, or 12 weeks). The 8 hour photoperiod was provided by a growth chamber maintained at a constant temperature of 20°C. Photoperiod in the chamber was provided by fluorescent and incandescent bulbs delivering a mean photosynthetic photon flux density (PPFD) of 161.4 ± 3.5 μmol m⁻² s⁻¹ at pot level. The 14 hour photoperiod was provided by a
natural daylight in a greenhouse supplemented with incandescent bulbs providing a mean PPFD of 269.3 ± 18.7 μmol m\(^{-2}\) s\(^{-1}\) at pot level. Mean greenhouse temperature during the experiment was 24.2 ± 0.2°C. All ramets exposed to a vernalization treatment were acclimated in a 13°C germination cabinet for 3 days prior to, and just after, the vernalization treatment. Plants were vernalized in the 6°C germination cabinet. This experiment was only conducted once as the core method was found to be poor for establishing new plants for experimental purposes relative to the root fragments or seeds used in subsequent experiments.

5.3.6. Experiment 2 - Effect of Vernalization and Photoperiod on Ramets Established from Root Fragments

The objective of this experiment was to determine the main and interactive effects of photoperiod and vernalization on the induction of flowering in red sorrel ramets established from creeping root fragments. Ramets were established from creeping roots collected from Mt. Thom, Nova Scotia, on November 2, 2011. For this experiment, 5 sprouted root fragments were planted to a depth of 2cm in 715cm\(^3\) plastic cell packs filled with a 2:1:1 mixture of sand, potting mix, and pro-mix. Each cell pack was considered an experimental unit, and 6 replicate cell packs were used for each treatment.

The experiment was designed using a 3 X 2 factorial treatment arrangement in a completely randomized design. Treatment factors were photoperiod (8hr, 16hr, or increasing) and vernalization (yes or no). The 8 hour photoperiod was obtained by enclosing a grow shelf in cardboard to prevent contamination of the experimental units with light from adjacent shelves. The increasing photoperiod simply consisted of growing ramets under an 8 hour photoperiod prior to transfer to the 16 hour photoperiod. Ramets exposed to an increasing photoperiod
without vernalization were simply transferred from the 8 hour to the 16 hour photoperiod on the date that ramets were transferred to the vernalization treatment. Ramets exposed to an increasing photoperiod with vernalization were transferred to the 16 hour photoperiod after the vernalization treatment. Ramets were grown for 16 weeks before initiation of the vernalization and increasing photoperiod treatments. All ramets exposed to a vernalization treatment were acclimated in a 15°C germination cabinet for 7 days prior to, and just after, the vernalization treatment. Plants were vernalized in the cold storage facility for 12 weeks. Ramets were classified as those having <5 leaves and those having ≥5 leaves during pre and post-vernalization ramet counts for this experiment due to the abundance of small ramets that had emerged in some cell packs prior to vernalization.

5.3.7. Experiment 3 - Effect of Vernalization Duration on Ramets Established from Root Fragments

The objective of this experiment was to determine the vernalization duration required to saturate the flowering response in red sorrel ramets established from creeping root fragments. Red sorrel ramets were established from roots collected from established field populations in a lowbush blueberry field in Pigeon Hill, Nova Scotia, on June 28, 2011. Roots were collected as outlined above but were not sprouted prior to planting. Three root fragments were planted to a depth of 2 cm in 715 cm³ plastic cell packs filled with a 1:1:1 mixture of sand, potting mix, and pro-mix. Each cell pack was considered an experimental unit, and 10 replicate cell packs were used for each treatment. The experiment was conducted on shelves in the growth facility and plants were maintained under a 16 hour photoperiod.
The experiment was a completely randomized design with six treatments. Treatments consisted of a control in which plants were grown under the 16 hour photoperiod for the duration of the experiment, and vernalization for durations of 2, 4, 6, 8, and 10 weeks. Plants were grown for 10 weeks prior to transfer to the vernalization treatment, and plants for each treatment were placed in the vernalization treatment at the same time. The vernalization treatment was conducted in the 6°C germination cabinet and plants were acclimated in a 13°C germination cabinet for 7 days prior to, and just after, the vernalization treatment.

5.3.8. Experiment 4 - Effect of Vernalization Duration on Plants Established from Seed

The objective of this experiment was to determine the vernalization duration required to saturate the flowering response of red sorrel plants established from seed. Red sorrel plants were established from seed collected from established field populations in a lowbush blueberry field in Pigeon Hill, Nova Scotia, in August 2011. Single sprouted seeds were planted to a depth of 2cm in 715cm³ plastic cell packs filled with a 2:1:1 mixture of sand, potting mix, and pro-mix. Each cell pack was considered an experimental unit, and 10 replicate cell packs were used for each treatment. The experiment was conducted on shelves in the growth facility and plants were maintained under a 16 hour photoperiod.

The experiment was a completely randomized design with 4 treatments. Treatments consisted of 1) control in which plants were grown under the 16 hour photoperiod for the duration of the experiment, and vernalization for durations of 5, 10, and 15 weeks. Plants were grown for 12 weeks prior to transfer to the vernalization treatment, and plants for each treatment were placed in the vernalization treatment at the same time. The vernalization treatment was conducted in the cold storage facility. All plants exposed to a vernalization treatment were
acclimated in a 15°C germination cabinet for 7 days prior to, and just after, the vernalization treatment. Additional data collection to that outlined above included counts of the number of flowering stems per flowering plant and the number of flowers per flowering stem.

5.3.9. Experiment 5 - Effect of Pre and Post-vernalization Ramet Removal Under Field Conditions

The objective of this experiment was to determine the effect of pre and post-vernalization ramet removal on flowering ramet density in field populations of red sorrel. Field sites were located at North River and Mt. Thom, Nova Scotia (Table 5.1). Sites were established at the end of the cropping year in 2010 but prior to the fall pruning operation. Therefore, all plots were pruned manually using a rotary mower in early October 2010. Plot size was 2 X 6m. Treatments were arranged in randomized complete block at each site and included 1) an untreated control, 2) Fall ramet removal with an application of paraquat, and 3) spring ramet removal with an application of paraquat. Each treatment was replicated four times. Paraquat was applied at a rate of 1100 g a.i. ha⁻¹ using a CO₂ pressurized research plot sprayer outfitted with Teejet XR 11002 nozzles calibrated to deliver a water volume of 400L ha⁻¹ at a pressure of 40 PSI. Fall and spring paraquat applications occurred on October 12, 2010 and May 13, 2011, respectively. Detailed recording of ramet dynamics and flowering in each plot was conducted in a single 30 X 30cm quadrat established near the center of each plot. New ramets were counted and marked with colored elastics at approximately 2 week intervals throughout the fall and spring. An initial ramet count was conducted in all treatments prior to the fall paraquat application. Dead ramets were counted and elastics were removed on each counting date during the fall and spring. New and dead ramet counts were used to determine the net ramet population in each quadrat during the fall and spring of the experiment. New ramets counted in the fall were always marked with the
Table 5.1. Description of study sites used to determine the effects of fall and spring red sorrel ramet removal on ramet dynamics and ramet flowering in lowbush blueberry.

<table>
<thead>
<tr>
<th>Site-Year</th>
<th>Production Year</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
<th>Soil Type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Soil pH&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Soil %OM&lt;sup&gt;y&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mount Thom-2011</td>
<td>Non-bearing</td>
<td>45°29’30.373” N</td>
<td>62°59’25.200” W</td>
<td>229</td>
<td>Sandy loam</td>
<td>4.8</td>
<td>7.4</td>
</tr>
<tr>
<td>North River-2011</td>
<td>Non-bearing</td>
<td>45°27’54.431” N</td>
<td>63°12’46.471” W</td>
<td>92</td>
<td>Silt loam</td>
<td>-&lt;sup&gt;x&lt;/sup&gt;</td>
<td>-&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>soil type for North River-2011 and Mount Thom-2011 obtained from Webb et al., 1991.

<sup>y</sup>pH and % OM (% organic matter) determined from 4 soil cores taken to a depth of 10 cm at each site. Cores were combined to form a composite sample for each site. Composite samples submitted to the Nova Scotia Department of Agriculture Provincial Analytical Laboratory for analysis.

<sup>x</sup>Soil pH and %OM data for this site were unavailable.
same colored elastics so that the fall ramet cohort could easily be kept separate from new ramets emerging in the spring in each quadrat. The density of flowering ramets in each quadrat was recorded throughout the spring of 2011, and the contribution of the overwintering and spring ramet cohorts to the total population of flowering ramets was determined based on the elastic color of flowering ramets. Ramets were counted as flowering when the pistils or stamens of open flowers were visible to the naked eye, and all male and female ramets were marked with colored paper clips at the time of flowering.

5.3.10. Experiments 6 and 7 - Effect of Pre and Post-vernalization Ramet Removal Under Controlled Conditions

The objective of these experiments were to determine the effects of pre and post-vernalization ramet removal on the density of flowering red sorrel ramets under controlled conditions. Red sorrel ramets were established from roots and seeds collected from established field populations in a lowbush blueberry field in Pigeon Hill, Nova Scotia. Roots were collected on June 28, 2011 and seeds were collected in August 2011. Roots and seeds were collected as outlined above, but roots were not sprouted prior to planting. Three root fragments were planted to a depth of 2cm in 715cm³ plastic cell packs filled with a 1:1:1 mixture of sand, potting mix, and pro-mix. Each cell pack was considered an experimental unit, and 7 replicate cell packs were used for each treatment. For the seed experiment, a single germinated seed was planted to a depth of 2cm in the center of 20cm diameter plastic pots with a volume of 3142 cm³. Pots were filled with a 2:1:1 mixture of sand, potting mix, and pro-mix. Each pot was considered an experimental unit, and 5 replicate pots were used for each treatment. Each experiment was conducted in the growth facility and plants were maintained under a 16 hour photoperiod.
The experiment was a completely randomized design with 4 treatments. Treatments consisted of 1) control in which plants were grown under the 16 hour photoperiod for the duration of the experiment, 2) Pre-vernalization ramet removal in which all above-ground plant material in each cell pack or pot was clipped at soil level prior to the vernalization treatment (Clip-Pre), 3) post-vernalization ramet removal in which all above-ground plant material in each cell pack or pot was clipped at soil level just after the vernalization treatment (Clip-Post), and 4) ramet vernalization with no clipping (Clip-No). Plants from seed and root fragments were grown for 16 weeks prior to transfer to the vernalization treatment. Plants grown from root fragments were vernalized in the 6°C germination cabinet for 16 weeks and plants grown from seed were vernalized in the cold storage facility for 12 weeks. Plants grown from root fragments and seed were acclimated in a 13 and 15°C germination cabinet, respectively, for 7 days prior to, and just after, the vernalization treatment. Plants subject to the pre-vernalization clipping treatment were clipped prior to transfer to the acclimation chamber. Plants subject to the post-vernalization clipping treatment were clipped after removal from the acclimation chamber following vernalization.

The total density of flowering ramets in each treatment was expressed as the proportion of the total ramet population that could potentially flower. Potential flowering ramet populations for the control, Clip-No, Clip-Pre, and Clip-Post treatments were the total density of ramets in each pot at the end of the experiment, the pre-vernalization ramet density, the post-vernalization ramet density, and the total number of ramets emerging following the post-vernalization clipping, respectively.
5.3.11. Experiment 8 - Effect of Decreasing Photoperiod and Decreasing Temperature Prior to Vernalization

The objective of this experiment was to determine if exposure of red sorrel ramets to decreasing photoperiod or temperature prior to vernalization increases the frequency of flowering ramets. Red sorrel ramets were established from creeping roots collected from established field populations in a lowbush blueberry field in Mt. Thom, Nova Scotia, in early September 2011. For this experiment, 4 sprouted root fragments were planted to a depth of 2cm in 715cm³ plastic cell packs filled with a 1.5:1:1 mixture of sand, potting mix, and pro-mix. Each cell pack was considered an experimental unit, and ten replicate cell packs were used for each treatment. After planting, all cell packs were transferred to the greenhouse for approximately 3 weeks prior to the assigning of experimental treatments outlined below. Cell packs were placed in the greenhouse on September 13, 2011, and treatments were randomly assigned to each cell pack on October 7, 2011.

The experiment was designed using a 2 X 2 X 2 factorial arrangement in a completely randomized design. Treatment factors were photoperiod (constant, decreasing), temperature (constant, decreasing), and vernalization (yes or no). Experimental treatments consisted of 1) a control in which ramets were grown for the duration of the experiment under constant 14 hour photoperiod (cP), constant temperature (cT), and no vernalization, 2) ramets grown under constant 14 hour photoperiod and constant temperature for 6 weeks prior to vernalization, 3) ramets grown under decreasing photoperiod (dP) and constant temperature for 6 weeks with no vernalization, 4) ramets grown under decreasing photoperiod and constant temperature for 6 weeks with no vernalization, 5) ramets grown under 14 hour photoperiod and decreasing temperature (dT) for 6 weeks with no vernalization, 6) ramets grown under 14 hour photoperiod and decreasing temperature (dT) for 6 weeks with no vernalization.
and decreasing temperature for 6 weeks prior to vernalization, 7) ramets grown under decreasing photoperiod and decreasing temperature for 6 weeks with no vernalization, and 8) ramets grown under decreasing photoperiod and decreasing temperature for 6 weeks prior to vernalization. For treatments 1 and 2 ramets simply emerged and were maintained under the initial greenhouse conditions described above for the duration of the experiment. For treatments 3 and 4 ramets were transferred to an alternate lighting system established in the same greenhouse and the photoperiod was decreased by 1 hour per week until ramets were exposed to an 8 hour photoperiod for 1 week prior to vernalization or transfer back to the 14 hour photoperiod. For treatments 5 through 8 ramets were transferred to an unheated greenhouse in which an identical lighting system to that established in the warm greenhouse had been established. Ramets in treatments 5 and 6 were exposed to a constant 14 hour photoperiod and the naturally decreasing temperatures during the 6 week period between October 7 and November 18 in the unheated greenhouse. Ramets in treatments 7 and 8 were exposed to the same decreasing photoperiod as already described for treatments 3 and 4, as well as the naturally decreasing temperatures in the unheated greenhouse. All ramets were transferred to their respective treatment locations when treatments were assigned on October 7, 2011. Decreasing photoperiod treatments were initiated immediately after ramets were moved, that is, ramets were transferred from a 14 hour to a 13 hour photoperiod on the date that treatments were assigned.

Mean daily temperature in the unheated greenhouse during the first week after ramets were transferred was $14.6 \pm 1.2^\circ C$ and declined to $14.2 \pm 0.9$, $9.8 \pm 1.0$, and $5.6 \pm 0.42^\circ C$ by the 2nd, 3rd, and 4th week after transfer, respectively. However, mild weather during the last 2 weeks of the decreasing temperature treatment resulted in an increase in greenhouse temperature to 9.3
± 1.3 and 10.1 ± 1.1°C during weeks 5 and 6 of the decreasing temperature treatments, respectively. However, these temperatures were still about 13°C lower than the warm greenhouse temperature and still allowed for exposure of ramets to decreasing temperature. All ramets were then moved back to the warm greenhouse under 14 hour photoperiod or to the vernalization treatment on November 18, 2011. Vernalization was conducted in the 6°C germination cabinet and plants were acclimated at 15°C for 7 days prior to, and just after, the vernalization treatment. Vernalized ramets were then transferred back to the constant 14 hour photoperiod in the warm greenhouse. In this experiment ramet density and leaf number were determined prior to transfer to the pre-vernalization stimulus treatments and prior to transfer to the vernalization treatment, but post-vernalization ramet counts were not conducted.

5.3.12. Statistical Analysis

Treatment effects on the frequency of cell packs or pots with flowering ramets were determined using categorical weighted-least-squares ANOVA in the PROC CATMOD procedure of SAS (SAS Version 9.3, SAS Institute, Cary, North Carolina). All factors were considered as categorical variables and were modeled as fixed effects in all analyses. Differences between treatments were determined by pairwise contrasts in PROC CATMOD. Due to the nature of the PROC CATMOD procedure, significant effects of experimental runs could not be determined. Therefore, data were combined across experimental runs for each analysis. However, differences in ramet density and ramet leaf number in all cell packs or pots prior to pre-vernalization stimulus or vernalization treatments were assessed across treatments and experimental runs by ANOVA (PROC GLM, SAS Version 9.3, SAS Institute, Cary, North Carolina) to determine any inherent differences in these parameters across treatments and experimental runs. In experiments with missing values the ANOVA was conducted using PROC
MIXED (SAS Version 9.3, SAS Institute, Cary, North Carolina). Data were transformed as required to meet the assumptions of the variance analysis. Arithmetic means are presented for transformed data. LSMEANS were determined for all data that did not require transformation prior to ANOVA. Mean flowering ramet leaf number, flowering ramet stem height, and flowering ramet survival data are presented for experimental treatments in which flowering occurred only. Means separation of these data were not attempted due to uneven sample sizes that occurred across treatments.

The number of flowers per flowering stem was related to flower stem height in the vernalization duration experiment using plants established from seed by a quadratic equation of the form

\[ y = a + bx + cx^2 \]  

where \( y \) is the number of flowers per flower stem, \( x \) is flower stem height, \( a \) is the intercept, and \( b \) and \( c \) are shape parameters. Parameters of the equation were estimated using the regression wizard function of SigmaPlot Version 12 for Windows (Systat Software Inc., Chicago, IL).

Net ramet populations in the control and paraquat treatments for the field ramet removal study were compared across treatments over the duration of the experiment using a repeated measures ANOVA in PROC MIXED (SAS Version 9.3, SAS Institute, Cary, North Carolina). A spatial power covariance structure was used in the analysis due to unequal spacing of counting dates (Bowley, 2008). Counting date was considered the repeated effect in the model, and the effects of treatment, site, and the subsequent interactions were modeled as fixed effects. Block was modeled as a random effect. Differences in mean net ramet density at each site were
determined by Tukey’s multiple means comparison test at the 0.05 level of significance. ANOVA (PROC MIXED, SAS Version 9.3, SAS Institute, Cary, North Carolina) was used to test the effects of treatment, site, and the treatment X site interaction effects on the density of flowering ramets in each treatment. All effects were considered fixed, and significance was based on the 0.05 probability level. Contrasts were used to estimate differences between the density of flowering ramets in the control and the ramet removal treatments, as well as to assess differences between the two timings of ramet removal used. An initial ANOVA (PROC MIXED, SAS Version 9.3, SAS Institute, Cary, North Carolina) was used to test the effects of treatment, site, and the treatment X site interaction effects on the percent contribution of the overwintering, May, June, and late summer (July – August) ramet cohorts to the total population of flowering ramets. All effects were treated as fixed, and contrasts were used to test for differences in percent contribution of each cohort between treatments. Data were LOG(X) or Squareroot(X) transformed as required to meet the assumptions of the variance analysis. Back-transformed means are presented for interpretation.

5.4. Results

5.4.1. Experiment 1 - Effect of Vernalization and Photoperiod on Ramets Established from Soil Cores

Mean pre-vernalization ramet density and leaf number in pots established from cores did not vary significantly across treatments (p≥0.4985 ) and averaged 4.3 ± 0.45 ramets pot⁻¹ and 7.8 ± 0.43 leaves ramet⁻¹, respectively. The initial PROC CATMOD ANOVA indicated significant effects of photoperiod, vernalization, and the photoperiod X vernalization interaction on the frequency of pots with flowering ramets (Table 5.2). No ramets flowered without vernalization. Vernalization for 12 weeks at 6°C induced flowering in 71% of the pots maintained under a pre
and post-vernalization photoperiod of 14 hours, and flowering ramets were observed in 29% of pots vernalized for 6 weeks at 6°C and maintained under a pre and post-vernalization photoperiod of 14 hours. Ramets maintained under a pre and post-vernalization photoperiod of 8 hours did not flower following either 6 or 12 weeks of vernalization, and ramets maintained under a pre-vernalization photoperiod of 8 hours did not flower when transferred to a post-vernalization photoperiod of 14 hours.

Table 5.2. Weighted-Least-Squares ANOVA of flower frequency in red sorrel ramets established from soil cores and exposed to short, long or increasing photoperiods with or without vernalization (CATMOD procedure of SAS; SAS Version 9.3, SAS Institute, Cary, North Carolina).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>1677.50</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>2</td>
<td>16.90</td>
<td>P=0.0002</td>
</tr>
<tr>
<td>Vernalization</td>
<td>2</td>
<td>19.26</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod X Vernalization</td>
<td>4</td>
<td>19.79</td>
<td>P=0.0006</td>
</tr>
<tr>
<td>Residual</td>
<td>0</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

5.4.2. Experiment 2 - Effect of Vernalization and Photoperiod on Ramets Established from Root Fragments

Mean pre and post-vernalization density of ramets with ≥ 5 leaves did not vary significantly by treatment (p≥0.0561) or the treatment X experimental run interaction (p≥0.3550) and averaged 2.3 ± 0.4 to 5.2 ± 0.6 ramets cell pack⁻¹ (Table 5.3). Mean leaf number of these ramets was also unaffected by treatment (p≥0.0826) or the treatment X experimental run interaction (p≥0.6196) and ranged from 12.6 ± 1.5 to 22.7 ± 3.5 leaves ramet⁻¹ (Table 5.3). Fewer ramets with leaf number < 5 were produced under short photoperiods (p<0.0001) but this effect did not vary across experimental runs (p≥0.3513) (Table 5.3). There was a significant effect of photoperiod, vernalization, and the photoperiod X vernalization interaction on the frequency of cell packs with
flowering ramets (Table 5.4). No flowering ramets were observed in cell packs that did not receive a vernalization treatment (Table 5.5). Frequency of cell packs with flowering ramets was highest (92%) in cell packs maintained under a pre and post-vernalization photoperiod of 16 hours (Table 5.5). Flowering ramets were observed in only 22% of the cell packs maintained under a pre-vernalization photoperiod of 8 hours but transferred to the 16 hour photoperiod following vernalization (Table 5.5). Both mean density of flowering ramets and the proportion of vernalized ramets flowering was highest in the cell packs exposed to a pre and post-vernalization photoperiod of 16 hours, and ramets bolted within about 15 days after transfer to the grow shelves (Table 5.5). The first flowering ramets were observed around 20 days (500 GDD) after transfer to the grow shelves, and 50 and 90% of ramets had flowered by 31 and 43 days (780 and 1074 GDD) after transfer to the grow shelves, respectively (Fig. 5.1). Peak flowering occurred around 50 days (1393 GDD) after transfer to the grow shelves (Fig. 5.1). Mean leaf number of flowering ramets was higher in cell packs exposed to increasing photoperiod following vernalization, but mean flower stem height was similar across treatments (Table 5.5). Mean density of vegetative ramets at the end of the experiment was highest in cell packs that did not receive a vernalization treatment, and cell packs kept in short photoperiods in combination with vernalization generally had fewer ramets but higher mean ramet leaf number (Table 5.6). Survival of flowering ramets ranged from 69-83% at the end of the experiment (Table 5.6).
Table 5.3. Mean pre and post-vernalization ramet density and ramet leaf number in various photoperiod treatments with and without vernalization.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-vern</th>
<th>Post-vern</th>
<th>Pre-vern</th>
<th>Post-vern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ramet Density of Ramets with Leaf # ≥ 5</td>
<td>Mean Leaf #</td>
<td>Ramet Density of Ramets with Leaf # &lt; 5</td>
<td>Mean Leaf #</td>
</tr>
<tr>
<td>P16:VN</td>
<td>5.0 ± 0.6 a</td>
<td>18.7 ± 2.3 a</td>
<td>10.8 ± 1.8 a</td>
<td>- x</td>
</tr>
<tr>
<td>P16:VY</td>
<td>5.2 ± 0.6 a</td>
<td>18.7 ± 1.6 a</td>
<td>9.3 ± 1.6 a</td>
<td>3.3 ± 0.4 a</td>
</tr>
<tr>
<td>P8:VN</td>
<td>3.3 ± 0.6 a</td>
<td>12.6 ± 1.5 a</td>
<td>0 ± 0 b</td>
<td>13.4 ± 1.2 a</td>
</tr>
<tr>
<td>P8:VY</td>
<td>4.3 ± 0.6 a</td>
<td>15.3 ± 1.3 a</td>
<td>1.4 ± 0.4 b</td>
<td>22.7 ± 3.5 a</td>
</tr>
<tr>
<td>P↑:VN</td>
<td>4.4 ± 0.5 a</td>
<td>17.1 ± 2.7 a</td>
<td>0.6 ± 0.3 b</td>
<td>22.2 ± 4.0 a</td>
</tr>
<tr>
<td>P↑:VY</td>
<td>2.8 ± 0.6 a</td>
<td>17.0 ± 2.8 a</td>
<td>0.4 ± 0.3 b</td>
<td>0.6 ± 0.3 b</td>
</tr>
</tbody>
</table>

V, vernalization (Yes or No); P, photoperiod; 16, 8, are photoperiod lengths of 16 and 8 hours; ↑ is an increasing photoperiod in which ramets were maintained under 8 hour photoperiod and then transferred to 16 hour photoperiod with or without vernalization.

x Mean post-vernalization ramet density and ramet leaf number were only determined in cell packs exposed to a vernalization treatment.

Table 5.4. Weighted-Least-Squares ANOVA of flower frequency in red sorrel ramets exposed to short, long, or increasing photoperiods with or without vernalization (CATMOD procedure of SAS; SAS Version 9.3, SAS Institute, Cary, North Carolina).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>1961.52</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>2</td>
<td>125.92</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Vernalization</td>
<td>1</td>
<td>49.68</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod X Vernalization</td>
<td>2</td>
<td>126.27</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>0</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>
Table 5.5. Percent of cell packs with flowering ramets, mean flowering ramet leaf number and flower stem height, and mean days to bolting of red sorrel ramets exposed to various combinations of photoperiod and vernalization.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell Packs with Flowering Ramets</th>
<th>Flowering Ramet Density</th>
<th>Mean Flowering Ramet Leaf Number</th>
<th>Mean Flower Stem Height</th>
<th>Proportion of Vernalized Ramets Flowering</th>
<th>Mean Time to First Ramet Bolting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (n=12)</td>
<td>ramets cell pack$^z$</td>
<td>leaf # ramet$^z$</td>
<td>cm</td>
<td>%</td>
<td>days$^w$</td>
</tr>
<tr>
<td>P16:VN</td>
<td>0b$^y$</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P16:VY</td>
<td>92a</td>
<td>4.4 ± 1.0</td>
<td>23.7 ± 2.6</td>
<td>8.1 ± 0.8</td>
<td>33.8 ± 7.9</td>
<td>14.7 ± 1.2</td>
</tr>
<tr>
<td>P8:VN</td>
<td>0b</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P8:VY</td>
<td>0b</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>P↑:VN</td>
<td>0b</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P↑:VY</td>
<td>22b</td>
<td>0.33 ± 0.3</td>
<td>59.0 ± 3.0</td>
<td>9.3 ± 1.6</td>
<td>13.3 ± 9.3</td>
<td>28.0 ± 7.8</td>
</tr>
</tbody>
</table>

$^z$V, vernalization (Yes or No); P, photoperiod; 16, 8, are photoperiod lengths of 16 and 8 hours; ↑ is an increasing photoperiod in which ramets were maintained under 8 hour photoperiod and then transferred to 16 hour photoperiod with or without vernalization.

$^y$Flowering frequencies followed by the same letter do not differ significantly according to pairwise contrasts conducted in PROC CATMOD (SAS Version 9.3, SAS Institute, Cary, North Carolina).

$^x$Only cell packs with flowering ramets are included in mean calculations

$^w$Days from time of transfer from post-vernalization acclimation treatment to the grow room shelves
Table 5.6. Mean final vegetative and flowering ramet density, ramet leaf number, and flowering ramet survival in cell packs exposed to various photoperiod and vernalization treatments.

| Treatment | Vegetative Ramets | | Flowering Ramets | | Mean Flowering Ramet Survival $^{\text{c}}$ |
|-----------|------------------|------------------|------------------|------------------|
|           | Mean Density     | Mean Leaf #      | Mean Density     | Mean Leaf # $^{\text{x}}$ |                  |
|           | Ramets cell pack $^{\text{i}}$ | Leaves ramet $^{\text{j}}$ | Ramets cell pack $^{\text{i}}$ | Leaves ramet $^{\text{j}}$ |                  |
| P16:VN    | 35.6 ± 6.6a $^{\text{a}}$ | 4.6 ± 0.4 | 0 | 0 | - |
| P16:VY    | 14.0 ± 2.2bc | 4.7 ± 0.3 | 3.1 ± 0.9 | 21.6 ± 5.0 | 68.9 ± 11.2 |
| P8:VN     | 34.3 ± 9.1ab | 8.9 ± 1.8 | 0 | 0 | - |
| P8:VY     | 6.0 ± 1.1c | 21.1 ± 4.0 | 0 | 0 | - |
| P$^{\text{↑}}$:VN | 31.8 ± 8.8ab | 5.2 ± 0.7 | 0 | 0 | - |
| P$^{\text{↑}}$:VY | 6.8 ± 0.9c | 11.7 ± 2.4 | 0.25 ± 0.2 | 29.8 ± 7.8 | 83.3 ± 16.7 |

$^{\text{a}}$V, vernalization (Yes or No); P, photoperiod; 16, 8, are photoperiod lengths of 16 and 8 hours; $^{\text{↑}}$ is an increasing photoperiod in which ramets were maintained under 8 hour photoperiod and then transferred to 16 hour photoperiod with or without vernalization.

$^{\text{b}}$Means followed by the same letter do not differ significantly according to a Tukeys test at the 0.05 level of significance.

$^{\text{c}}$Only cell packs with flowering ramets were included in mean calculations.
Fig. 5.1. Mean percent cumulative flowering red sorrel ramets in cell packs in the P16:VY treatment in the photoperiod X vernalization experiment after transfer to grow shelves following vernalization for 12 weeks at 4.5 ± 0.1°C. DAT; Days after transfer to growth facility; GDD, growing degree-days ($T_{\text{base}}=0^\circ\text{C}$).
5.4.3. Experiment 3 - Effect of Vernalization Duration on Ramets Established from Root Fragments

Pre-vernalization ramet density and ramet leaf number averaged 4.2 ± 0.43 ramets cell pack\(^{-1}\) and 14.9 ± 1.0 leaves ramet\(^{-1}\), respectively, but did not vary significantly by treatment (p=0.1616) or the treatment X experimental run interaction (p=0.4912). However, none of the ramets vernalized for 0, 2, 4, 6, 8, or 10 weeks at 6\(^\circ\)C flowered.

5.4.4. Experiment 4 - Effect of Vernalization Duration on Plants Established from Seed

There was no significant effect of treatment (p≥0.3606) or the treatment X experimental run interaction (p≥0.1008) on mean pre or post-vernalization seed plant leaf number, ramet density, and ramet leaf number. Pre and post-vernalization seed plant leaf number averaged 52.4 ± 1.2 and 42.9 ± 1.45 leaves plant\(^{-1}\), respectively. Pre and post-vernalization ramet density in each cell pack averaged 5.9 ± 0.52 and 11.16 ± 0.91 ramets cell pack\(^{-1}\), respectively. Pre and post-vernalization ramet leaf number averaged 1.9 ± 0.09 and 2.13 ± 0.08 leaves ramet\(^{-1}\), respectively. The effect of vernalization duration on the frequency of cell packs with flowering seed plants or ramets was significant (Table 5.7), with the highest flowering frequency occurring at vernalization durations of 10 and 15 weeks (Table 5.8). Mean leaf and flower stem number for flowering seed plants were similar across treatments, but mean flowering stem height was shorter in flowering seed plants following 5 weeks of vernalization as compared to 10 and 15 weeks of vernalization (Table 5.8). The number of flowers per flowering stem increased in a non-linear fashion with flower stem height, but did not reach a maximum under the conditions of this experiment (Fig. 5.2). Flowering seed plants and ramets bolted sooner following 10 and 15 weeks of vernalization as compared to 5 weeks of vernalization (Table 5.8), which generally
Table 5.7. Weighted-Least-Squares ANOVA of flower frequency in red sorrel seed plants exposed to different vernalization durations (CATMOD procedure of SAS; SAS Version 9.3, SAS Institute, Cary, North Carolina).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>1069.31</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Vernalization Duration</td>
<td>3</td>
<td>75.91</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>0</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Table 5.8. Flowering frequency, mean time to seed plant and ramet bolting, mean density of flowering ramets, and mean flowering seed plant leaf and stem number in cell packs exposed to vernalization at 4.5 ± 0.1°C for durations of 0, 5, 10, and 15 weeks.

<table>
<thead>
<tr>
<th>Vernalization Duration</th>
<th>Cell Packs with Flowering Seed Plants and/or Ramets</th>
<th>Mean Flowering Seed Plant Leaf #&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Mean Flowering Seed Plant Stem #&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Mean Flowering Seed Plant Stem Height #&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Mean Time to Seed Plant Bolting #&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Mean Time to Ramet Bolting #&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Mean Density of Flowering Ramets</th>
</tr>
</thead>
<tbody>
<tr>
<td>weeks</td>
<td>% (n=20)</td>
<td>leaves plant #&lt;sup&gt;x&lt;/sup&gt;</td>
<td>stems plant #&lt;sup&gt;x&lt;/sup&gt;</td>
<td>cm</td>
<td>days #&lt;sup&gt;x&lt;/sup&gt;</td>
<td>days #&lt;sup&gt;x&lt;/sup&gt;</td>
<td>ramets cell pack #&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.0 ± 4.8</td>
<td>3.0 ± 1.0</td>
<td>6.3 ± 1.0</td>
<td>22</td>
<td>32</td>
<td>0.1 ± 0.07</td>
</tr>
<tr>
<td>10</td>
<td>65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.1 ± 4.8</td>
<td>4.0 ± 0.5</td>
<td>14.6 ± 0.9</td>
<td>14.6 ± 1.0</td>
<td>19.3 ± 3.4</td>
<td>0.9 ± 0.35</td>
</tr>
<tr>
<td>15</td>
<td>65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.6 ± 5.2</td>
<td>3.1 ± 0.4</td>
<td>16.6 ± 2.2</td>
<td>16.6 ± 2.3</td>
<td>13.0 ± 1.3</td>
<td>1.2 ± 0.44</td>
</tr>
</tbody>
</table>

<sup>Flowering frequencies followed by the same letter do not differ significantly according to pairwise contrasts conducted in PROC CATMOD (SAS Version 9.3, SAS Institute, Cary, North Carolina).

<sup>Only cell packs with flowering ramets are included in mean calculations</sup>

<sup>Days from time of transfer from post-vernialization acclimation treatment to the grow room shelves</sup>
Fig. 5.2. Quadratic relationship between red sorrel flower number per stem and height of the flowering stem. Symbols are observed data. The line is a quadratic equation of the form $y = 0.9411(11.4290) + 1.8883(1.6342)x + 0.1991(0.0503)x^2$. Parameters were estimated using the regression wizard function in Sigma Plot. SE of each parameter estimate is provided in parentheses.
resulted in earlier flowering in these treatments (Fig. 5.3A). Flowering seed plants and ramets were first observed in cell packs following 5 weeks of vernalization around 25 days (634 GDD) after transfer to the grow shelves (Fig. 5.3A), whereas plants grown from seed and ramets began to flower within about 14 days (348-366 GDD) after transfer following vernalization for 10 and 15 weeks (Fig. 5.3A). The last cell pack with a flowering plant grown from seed or ramet was observed 39 days (975 GDD) after transfer to the grow shelves following 5 weeks of vernalization (Fig. 5.3A). About half of the cell packs containing flowering plants grown from seed or ramets were observed around 20 days (521-542 GDD) after transfer following vernalization for 10 and 15 weeks, respectively, and the last cell packs with flowering plant grown from seed or ramets in these treatments were observed between 35 and 49 days (881-1237 GDD) after transfer (Fig. 5.3A). Cumulative ramet flowering began between 468 and 521 GDD after transfer to the grow shelves (Fig. 5.3B). Ramets reached 50% flowering between 663 and 712 GDD and flowering peaked between 1500 and 1700 GDD after transfer to the grow shelves (Fig. 5.3B). Survival of the initial plants grown from seed generally increased with increasing vernalization duration, though a large ramet population had become established in all treatments by the end of the experiment (Table 5.9). Survival of flowering ramets ranged from 12 to 100% by the end of the experiment (Table 5.9).
Table 5.9. Final seed plant survival, seed plant leaf number, ramet density, and ramet leaf number in cell packs exposed to vernalization at 4.5 ± 0.1°C for durations of 0, 5, 10, and 15 weeks.

<table>
<thead>
<tr>
<th>Vernalization Duration</th>
<th>Survival of Initial Seed Plants %</th>
<th>Mean Surviving Seed Plant Leaf # leaves plant⁻¹</th>
<th>Mean Vegetative Ramet Density ramets cell pack⁻¹</th>
<th>Mean Vegetative Ramet Leaf # leaves ramet⁻¹</th>
<th>Mean Survival of Flowering Ramets %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>10</td>
<td>32.5 ± 23.5</td>
<td>36.2 ± 7.8</td>
<td>3.8 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>38.1 ± 7.2</td>
<td>54.2 ± 16.3</td>
<td>3.3 ± 0.2</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>30.8 ± 10.0</td>
<td>27.7 ± 4.2</td>
<td>2.7 ± 0.1</td>
<td>12</td>
</tr>
<tr>
<td>15</td>
<td>90</td>
<td>46.2 ± 6.6</td>
<td>28.3 ± 4.3</td>
<td>3.4 ± 0.2</td>
<td>70</td>
</tr>
</tbody>
</table>
Fig. 5.3. A) Number of cell packs with flowering seed plants or ramets after transfer to grow shelves following 5, 10, or 15 weeks of vernalization at 4.5 ± 0.1°C, and B) Mean percent cumulative flowering ramets in cell packs following 5, 10, or 15 weeks of vernalization at 4.5 ± 0.1°C. DAT; Days after transfer to growth facility; GDD, growing degree-days (T_{base}=0°C) after transfer to growth facility.
There was a significant treatment X site interaction effect (p<0.0001) on net ramet density in each treatment. Therefore, net ramet density was analyzed separately at each site. There was a significant treatment X day interaction effect on net ramet density at each site (p≤0.0003). Ramet density was similar in all treatments at the time of establishment of the experiment (Fig. 5.4). Fall and spring paraquat applications significantly reduced net ramet densities at each site (Fig. 5.4), though ramet populations in the Fall paraquat treatment had begun to recover by late spring at Mt. Thom (Fig. 5.4A). There was no treatment X site interaction effect on the density of flowering ramets (p=0.9332) or the percent contribution of the overwintering and monthly ramet cohorts to the population of flowering ramets (p≥0.1079). These data were therefore combined across sites for analysis. The density of flowering ramets was significantly lower in the removal treatments when compared to the control treatment (Table 5.10). Timing of ramet removal did not have a significant effect on the density of flowering ramets, but both fall and spring paraquat applications reduced the contribution of the overwintering cohort to the population of flowering ramets (Table 5.10). Spring paraquat applications provided a greater reduction in the contribution of ramets emerging in May to the population of flowering ramets, but contributions from ramets emerging in June tended to be higher in this treatment (Table 5.10).
Table 5.10. Flowering red sorrel ramet density and percent contribution of emerged red sorrel ramets to flowering ramet populations following Fall and Spring application of paraquat in two lowbush blueberry fields in Nova Scotia, Canada. Flowering ramet density was subject to LOG(X) transformation to meet the assumptions of the variance analysis. Back-transformed means with 95% confidence limits are presented.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flowering Ramets</th>
<th>Contribution of Emerged Ramets to Flowering Ramet Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ramets m⁻²</td>
<td>Overwintering</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>1.Control</td>
<td>78 (22, 271)</td>
<td>61 ± 10.8</td>
</tr>
<tr>
<td>2.Fall Paraquat</td>
<td>18 (4, 63)</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>3.Spring Paraquat</td>
<td>7 (1, 25)</td>
<td>15 ± 12</td>
</tr>
</tbody>
</table>

Contrast Analysis

2,3 versus 1 **
2 versus 3 NS

NS, Not significant; ** significant at P<0.05 and P<0.01, respectively; CL, Confidence Limit.
²Ramets emerging in July and August.
Fig. 5.4. Repeated measures ANOVA of mean net red sorrel ramet density in lowbush blueberry following Fall and Spring paraquat applications at A) Mt. Thom, and B) North River, Nova Scotia in 2010 and 2011. Mean net ramet densities on each counting date that share the same letter do not differ significantly according to a Tukey’s multiple means comparison at the 0.05 level of significance. Data were LOG(x) transformed to meet the assumptions of the variance analysis. Back-transformed means are presented.
5.4.6. Experiments 6 and 7 - Effect of Pre and Post-vernalization Ramet Removal Under Controlled Conditions

There was a significant treatment X experimental run interaction effect on post-vernalization density of ramets with less than 5 leaves \((p=0.0033)\), therefore pre and post-vernalization ramet counts are presented separately for each experimental run in the experiment using ramets established from root fragments (Table 5.11). Pre-vernalization ramet counts did not vary significantly across treatments (Table 5.11). Post-vernalization counts of ramets with more than 5 leaves could not be made to conform to the assumptions of the variance analysis, so means for each treatment are presented. Density of larger ramets was highest in the control treatment in both runs, and no large ramets were found in treatments in which all ramets were clipped prior to the vernalization treatment (Table 5.11). Density of ramets with less than 5 leaves varied significantly across treatments in run 1 \((p=0.0111)\) but not in run 2 \((p=0.5210)\). Density of small ramets was highest in the vernalized treatments in run 1, particularly in the treatment in which ramets were clipped prior to vernalization (Table 5.11). This trend, however, did not persist in the second run of the experiment (Table 5.11). None of the ramets established from root fragments flowered in any of the treatments (Table 5.11).

There was no significant effect of treatment \((p\geq0.1672)\) or the treatment X experimental run interaction \((p\geq0.1787)\) on mean pre-vernalization seed plant leaf number, mean ramet density, or mean ramet leaf number in the ramet removal experiment using plants established from seed (Table 5.12). Seed plants in the pre-vernalization clipping treatment had re-sprouted an average of 12 ± 3 leaves during the 7-day acclimation period prior to transfer to the vernalization conditions. An average of 28 ± 6 ramets had emerged during this period as well, with a mean leaf number of 2 ± 0.1. Post-vernalization seed plant leaf number varied
Table 5.11. Mean pre and post-vernalization ramet counts and flowering frequency following pre and post vernalization clipping treatments applied to ramets established from planted root fragments.

<table>
<thead>
<tr>
<th>Experimental Run</th>
<th>Treatment</th>
<th>Pre-vernalization Counts</th>
<th>Post-vernalization Ramet Counts</th>
<th>Frequency of Cell Packs with Flowering Ramets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ramets with &gt; 5 Leaves</td>
<td>Ramets with ≤ 5 Leaves</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramets cell pack⁻¹</td>
<td>Ramets cell pack⁻¹</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>2.0 ± 0.4a *</td>
<td>18.0 ± 5.7a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clip-No</td>
<td>1.3 ± 0.2a</td>
<td>9.0 ± 4.2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clip-Pre</td>
<td>2.4 ± 0.5a</td>
<td>18.4 ± 3.3a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clip-Post</td>
<td>1.6 ± 0.3a</td>
<td>16.3 ± 7.8a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>2.7 ± 0.6a</td>
<td>11.3 ± 3.1a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clip-No</td>
<td>2.6 ± 0.6a</td>
<td>13.7 ± 2.8a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clip-Pre</td>
<td>3.3 ± 0.9a</td>
<td>12.4 ± 2.5a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clip-Post</td>
<td>3.6 ± 0.8a</td>
<td>12.4 ± 3.6a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramets cell pack⁻¹</td>
<td>Ramets cell pack⁻¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.1 ± 2.4</td>
<td>7.7 ± 4.6b</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3 ± 0.4</td>
<td>12.7 ± 4.5ab</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 ± 0</td>
<td>21.3 ± 2.5a</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3 ± 0.2</td>
<td>19.4 ± 4.3a</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.0 ± 1.4</td>
<td>12.6 ± 3.0a</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4 ± 0.7</td>
<td>10.7 ± 1.8a</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 ± 0</td>
<td>8.4 ± 2.7a</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.9 ± 0.9</td>
<td>8.4 ± 1.4a</td>
<td>0</td>
</tr>
</tbody>
</table>

*Means followed by the same letter do not differ significantly according to a Tukey’s multiple means comparison at the 0.05 level of significance. Means displayed determined using the LSMEANS statement in PROC MIXED.

Table 5.12. Mean pre and post-vernalization seed plant leaf number, ramet density, and ramet leaf number for red sorrel plants established from seed and subject to pre and post-vernalization clipping treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-vernalization Counts</th>
<th>Post-Vernalization Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed Plant Leaf #</td>
<td>Ramet Density</td>
</tr>
<tr>
<td></td>
<td>leaves plant⁻¹</td>
<td>ramets pot⁻¹</td>
</tr>
<tr>
<td>Control</td>
<td>141 ± 16a</td>
<td>72 ± 10a</td>
</tr>
<tr>
<td>Clip-No</td>
<td>148 ± 15a</td>
<td>55 ± 9a</td>
</tr>
<tr>
<td>Clip-Pre</td>
<td>132 ± 10a</td>
<td>48 ± 9a</td>
</tr>
<tr>
<td>Clip-Post</td>
<td>153 ± 13a</td>
<td>73 ± 9a</td>
</tr>
</tbody>
</table>

*Means followed by the same letter do not differ significantly according to a Tukey’s multiple means comparison at the 0.05 level of significance. Means displayed determined using the LSMEANS statement in PROC MIXED.

*Control plants not included in post-vernalization seed plant leaf and ramet counts.
significantly across treatment (p=0.0003) but was not affected by the treatment X experimental run interaction (p=0.6518). Post-vernalization seed plant leaf number was significantly reduced in the Clip-Pre treatment relative to the Clip-No and Clip-Post treatments (Table 5.12). Post-vernalization ramet density and ramet leaf number were not affected by treatment (p≥0.0860) or the treatment X experimental run interaction (p≥0.2118) (Table 5.12). The frequency of pots with flowering seed plants or ramets was significantly affected by clipping treatment (Table 5.13), with a significant reduction in flowering frequency following pre and post-vernalization clipping (Table 5.14). Seed plants in the Clip-No treatment had a higher number of flower stalks, and both flower ramet density and proportion of ramets flowering were higher in pots that were not clipped (Table 5.14). Seed plants and ramets in the Clip-No treatment had begun to flower within 15 days (385 GDD) after transfer to the grow shelves, and all pots contained flowering seed plants or ramets by 37 days (908 GDD) after transfer (Fig. 5.5A). Approximately 90% of the flowering ramets had flowered by this time as well, with the additional 10% flowering between 37 and 86 days (908 and 2097 GDD) after transfer to the growth shelves (Fig. 5.5B).

Table 5.13. Weighted-Least-Squares ANOVA of flower frequency in red sorrel seed plants and ramets exposed to pre and post-vernalization clipping treatments (CATMOD procedure of SAS; SAS Version 9.3, SAS Institute, Cary, North Carolina).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>680.02</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Clipping Treatment</td>
<td>3</td>
<td>149.70</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>0</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>
Table 5.14. Effect of pre and post-vernalization clipping of red sorrel ramets and seed plants on flower frequency, flower stem density, density of flowering ramets, and proportion of ramets flowering.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pots with Flowering Seed Plant or Ramets</th>
<th>Flowering Seed Plant Flower Stem Density</th>
<th>Density</th>
<th>Proportion of Potential Population That Flowered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10b²</td>
<td>2</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Clip-No</td>
<td>100a</td>
<td>9 ± 2</td>
<td>26 ± 8</td>
<td>44 ± 11</td>
</tr>
<tr>
<td>Clip-Pre</td>
<td>20b</td>
<td>3 ± 2</td>
<td>0.7 ± 0.5</td>
<td>0.8 ± 0.7</td>
</tr>
<tr>
<td>Clip-Post</td>
<td>30b</td>
<td>-</td>
<td>0.4 ± 0.22</td>
<td>0.6 ± 0.3</td>
</tr>
</tbody>
</table>

²Flowering frequencies followed by the same letter do not differ significantly according to pairwise contrasts conducted in PROC CATMOD (SAS Version 9.3, SAS Institute, Cary, North Carolina).

³Only pots with flowering seedplants or ramets are included in mean calculations; means with no SE indicate only one pot in which flowering occurred; - indicates no flowering seed plants.

³Potential population of flowering ramets was calculated as follows; Control, total number of ramets per pot at the end of the experiment; Clip-No, Pre-vernalization ramet density in each pot; Clip-Pre, Post-vernalization ramet density in each pot; Clip-Post, total number of ramets per pot at the end of the experiment.
Fig. 5.5. A) Number of pots with a flowering red sorrel seed plant or ramet and B) percent cumulative flowering ramets in the Clip-No treatment of the pre and post-vernalization ramet removal experiment following transfer to a 24.4 ± 0.2°C growth facility after vernalization at 4.5 ± 0.1°C for 12 weeks. DAT; Days after transfer to growth facility; GDD, growing degree-days (T_{base}=0°C) after transfer to growth facility.
5.4.7. Experiment 8 - Effect of Decreasing Photoperiod and Decreasing Temperature Prior to Vernalization

There were no significant effects of treatment (p≥0.0670) or the treatment X experimental run interaction (p≥0.3217) on mean ramet density and ramet leaf number in cell packs prior to transfer to pre-vernalization stimulus or vernalization treatments. Ramet density and ramet leaf number averaged 4.2 ± 0.17 ramets cell pack\(^{-1}\) and 2.9 ± 0.08 leaves ramet\(^{-1}\) prior to transfer to the pre-vernalization stimulus treatments. Ramet density and ramet leaf number averaged 4.9 ± 0.19 ramets cell pack\(^{-1}\) and 11.7 ± 0.44 leaves ramet\(^{-1}\) at the initiation of the vernalization treatment. The initial PROC CATMOD ANOVA indicated no significant effect of temperature or the temperature X photoperiod interaction on the frequency of cell packs with flowering ramets (Table 5.15). Therefore, flowering data were combined across temperature treatments for the analysis. There was a significant photoperiod X vernalization interaction effect on flowering frequency (Table 5.16) with significantly higher flowering frequency in cell packs exposed to decreasing photoperiod prior to vernalization (Table 5.17). Density of flowering ramets and proportion of vernalized ramets flowering were higher in cell packs exposed to decreasing photoperiod prior to vernalization, and these ramets bolted earlier than ramets exposed to a constant photoperiod prior to vernalization (Table 5.17). Despite this earlier bolting, flowering ramets were observed in both treatments by 10 days (273 GDD) after transfer to the greenhouse (Fig. 5.6). However, ramets exposed to decreasing photoperiod prior to vernalization flowered at a faster rate and reached 50 and 90% flowering about 9 days (224-227 GDD) earlier than ramets exposed to constant photoperiod prior to vernalization (Fig. 5.6). Peak flowering generally occurred in both treatments between 53 and 60 days (1300-1500GDD) after transfer to the greenhouse (Fig. 5.6).
Mean leaf number and flower stem height were similar in all flowering ramets, irregardless of photoperiod treatment prior to vernalization (Table 5.17). Unvernalized cell packs for each respective photoperiod treatment contained about twice as many vegetative ramets by the end of the experiment when compared to cell packs exposed to the vernalization treatment (Table 5.18). Mean density of flowering ramets in the vernalization treatments was <1 ramet cell pack\(^{-1}\) by the end of the experiment, and survival of flowering ramets averaged about 41% in each photoperiod treatment exposed to vernalization (Table 5.18).
Table 5.15. Weighted-Least-Squares ANOVA for the saturated model for frequency of cell packs with flowering red sorrel ramets following exposure to constant or decreasing photoperiod and constant or decreasing temperature, with or without vernalization for 16 weeks at 6°C (CATMOD procedure of SAS; SAS Version 9.3, SAS Institute, Cary, North Carolina).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>2731.83</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>1</td>
<td>20.85</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>0.00</td>
<td>1.0000</td>
</tr>
<tr>
<td>Photoperiod X Temperature</td>
<td>1</td>
<td>0.00</td>
<td>1.0000</td>
</tr>
<tr>
<td>Vernalization</td>
<td>1</td>
<td>135.93</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod X Vernalization</td>
<td>1</td>
<td>20.85</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Temperature X Vernalization</td>
<td>1</td>
<td>0.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Photoperiod X Temperature X Vernalization</td>
<td>1</td>
<td>0.00</td>
<td>1.000</td>
</tr>
<tr>
<td>Residual</td>
<td>0</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

Table 5.16. Weighted-Least-Squares ANOVA for the reduced model for frequency of cell packs with flowering red sorrel ramets following exposure to constant or decreasing photoperiod, with or without vernalization for 16 weeks at 6°C (CATMOD procedure of SAS; SAS Version 9.3, SAS Institute, Cary, North Carolina).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>2734.81</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>1</td>
<td>20.88</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Vernalization</td>
<td>1</td>
<td>136.22</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Photoperiod X Vernalization</td>
<td>1</td>
<td>20.88</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>0</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>
Table 5.17. Frequency of cell packs with flowering red sorrel ramets, mean density, leaf number, stem height, and time to bolting of flowering ramets, and proportion of vernalized ramets flowering, in cell packs exposed to constant or decreasing photoperiod, with or without vernalization for 16 weeks at 6°C.

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;z&lt;/sup&gt;</th>
<th>Cell Packs with Flowering Ramets</th>
<th>Flowering Ramet Density</th>
<th>Mean Flowering Ramet Leaf #&lt;sup&gt;x&lt;/sup&gt;</th>
<th>Mean Flower Stem Height&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Proportion of Vernalized Ramets Flowering</th>
<th>Mean Time to First Ramet Bolting&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>cP, VN</td>
<td>% (n=40) ramets cell pack&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;-&lt;/sup&gt;</td>
<td>-</td>
<td>21 ± 5.7</td>
<td>21.8 ± 3.1</td>
</tr>
<tr>
<td>cP, VY</td>
<td>35b</td>
<td>0.8 ± 0.3</td>
<td>26.2 ± 3.3</td>
<td>12.2 ± 1.4</td>
<td>0.5 ± 0.2</td>
<td>16.0 ± 5.6</td>
</tr>
<tr>
<td>dP, VN</td>
<td>0c</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>dP, VY</td>
<td>80a</td>
<td>2.7 ± 0.3</td>
<td>24.2 ± 1.5</td>
<td>13.3 ± 0.4</td>
<td>0.9 ± 0.2</td>
<td>13.3 ± 2.3</td>
</tr>
</tbody>
</table>

<sup>z</sup>cP, constant photoperiod; cT, constant temperature; dP, decreasing photoperiod; dT, decreasing temperature.

<sup>y</sup>Flowering frequencies followed by the same letter do not differ significantly according to pairwise contrasts conducted in PROC CATMOD (SAS Version 9.3, SAS Institute, Cary, North Carolina).

<sup>x</sup>Only cell packs with flowering ramets are included in mean calculations.

Table 5.18. Final vegetative and flowering ramet density, ramet leaf number, and flowering ramet survival in cell packs exposed to constant or decreasing photoperiod with or without vernalization for 16 weeks at 6°C.

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;z&lt;/sup&gt;</th>
<th>Vegetative Ramets</th>
<th>Flowering Ramets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Density</td>
<td>Mean Leaf #</td>
</tr>
<tr>
<td></td>
<td>ramets cell pack&lt;sup&gt;1&lt;/sup&gt;</td>
<td>leaves ramet&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>cP, VN</td>
<td>57.3 ± 5.2</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>cP, VY</td>
<td>32.2 ± 2.4</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>dP, VN</td>
<td>49.3 ± 5.8</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>dP, VY</td>
<td>23.7 ± 2.4</td>
<td>2.9 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>z</sup>cP, constant photoperiod; cT, constant temperature; dP, decreasing photoperiod; dT, decreasing temperature.

<sup>y</sup>Only cell packs with flowering ramets are included in mean calculations.
Fig. 5.6. Mean percent cumulative flowering of ramets in cell packs after transfer to the greenhouse following exposure to constant (n=14) or decreasing (n=32) photoperiod and vernalization for 16 weeks at 6°C. DAT; Days after transfer to greenhouse; GDD, growing degree-days (Tbase=0°C) after transfer to greenhouse.
5.5. Discussion

Red sorrel ramets propagated from creeping roots collected from established ramet populations in lowbush blueberry fields in Nova Scotia had an obligate requirement for vernalization to flower. The requirement was also predominant in plants established from seed; only one plant out of 140 plants established from seed flowered without vernalization. These data indicate that a vernalization requirement for flowering is a major component of the genetic structure of established red sorrel populations in lowbush blueberry fields in Nova Scotia. This has not been previously reported for red sorrel and provides a new understanding of the role of vernalization in ramet development of a polycarpic herbaceous creeping perennial.

The experiments on the interaction between photoperiod and vernalization provided strong insight into the effects of these factors on red sorrel ramet development in lowbush blueberry. Plants that require vernalization usually require a cold treatment followed by an inductive photoperiod for flowering to occur (Amasino, 2005). Red sorrel ramets required vernalization and subsequent exposure to long days for flower induction, similar to that reported for some monocarpic perennial species that also require vernalization to flower (Kachi and Hirose, 1983; Klinkhamer et al., 1987b). Evans (1971) indicated that long days are often required for flower induction in plants that require vernalization, and the results are in general agreement with other studies in which red sorrel has been classified as a long day plant (Carlson, 1965; Listowski and Jackowska, 1964). Flowering frequency was low when ramets were maintained under short photoperiods prior to vernalization (Table 5.5); ramets therefore appear to require growth under long photoperiods before chilling in order to confer flowering competency. The
majority of species studied for vernalization effects on flowering are maintained under long photoperiods prior to vernalization, primarily based on the assumption of emergence and establishment of plants early in the first season of growth. Red sorrel ramets, however, exhibit season long emergence (Chapters 3 and 4) and thus have vegetative ramets emerging under a variety of photoperiods during a given year. Our data would suggest that ramets must emerge and grow under a critical photoperiod for at least some portion of the growing season prior to chilling in order to obtain flowering competency. Cell packs maintained under the 8-hour photoperiod contained a similar number of large ramets and therefore should have been equally responsive to a vernalization treatment when compared to cell packs maintained under the 16 hour photoperiod (Table 5.3). Therefore, the data in this study point to a critical daylength prior to vernalization as the limiting factor for flower induction in red sorrel ramets.

Red sorrel ramets maintained under short photoperiods did not flower when transferred to long photoperiods without vernalization (Table 5.5). Wellensiek (1953, 1960) demonstrated that growth under short days can provide a partial substitution for vernalization in the monocarpic perennial plant *Campanula medium*, a phenomenon that may help explain the flowering of newly emerging red sorrel ramets in early spring under field conditions (Chapter 3). Chouard (1960) reported similar behavior in the perennial plant *Scabiosa succisa*, and recent work has also demonstrated a similar response in photoperiod sensitive accessions of winter wheat (Dubcovsky et al., 2006) and some temperate grasses (Heide, 1994). This response in plants, however, is variable (Chouard, 1960) and it would appear that red sorrel ramets are unable to utilize short photoperiods as a substitute for vernalization to confer flowering competence.
Although ramets appear to require growth under long photoperiods prior to vernalization to confer flowering competency, exposure of red sorrel ramets to decreasing photoperiod prior to vernalization did increase flowering frequency in the pre-vernalization stimulus experiment (Table 5.17). Thus, it appears that ramets respond to, but do not have an absolute requirement for, pre-vernalization stimuli such as decreasing photoperiod. Similarly, Medd and Lovett (1978) found that Carduus nutans plants established under long days but exposed to short photoperiods prior to vernalization required shorter durations of exposure to cold temperatures for saturation of the vernalization response. C. nutans plants grown under long days prior to vernalization could still be induced to flower; the duration of cold treatment required to saturate the flowering response was simply longer. Similar responses have also been found in both the ornamental plant Hatiora gaertneri and the food crop Allium fistulosum L. (Rohwer and Heins, 2007; Yamasaki et al., 2000). It should be noted that flower stem height was similar for flowering ramets in both of the vernalization treatments (Table 5.17), indicating a similar flowering response at the ramet level despite lower flowering frequency in cell packs maintained under constant photoperiod prior to vernalization.

Decreasing temperature, rather than photoperiod, prior to vernalization is required for flowering in Euphorbia esula (Foley et al., 2009). Red sorrel ramets did not respond similarly in this study. The decreasing temperature treatment, however, was dependent upon the natural decrease in temperature observed during the autumn in Nova Scotia. While temperatures did decline during most of the pre-vernalization stimulus portion of the experiment, there was a small increase in mean daily temperature during the final two weeks of the pre-vernalization stimulus treatments. The true effect of decreasing temperature may therefore have been
confounded and this effect should be investigated further. Changes in photoperiod are often considered a more reliable environmental signal of impending seasonal change than temperature though (Battey, 2000; Heide, 2001; Minorsky, 2002; Searle and Coupland, 2004). For example, Kasperbauer et al. (1962) reported rapid increases in root biomass and root crown bud density of *Melilotus albus* (alba) and *M. officinalis* (L.) under the decreasing photoperiods associated with autumn in Iowa, regardless of temperature. Similarly, red sorrel plants develop more adventitious root buds under short photoperiods (Carlson, 1965). Sprouting capacity of creeping roots of *Sonchus arvensis* is also regulated more strongly by photoperiod than by temperature (Liew et al., 2012). It may be that decreasing photoperiod, rather than decreasing temperature, prior to vernalization is more important for red sorrel ramet development under field conditions in Nova Scotia. The role of pre-vernalization stimuli appears to be species-specific in the few species in which this phenomenon has been studied and more research in this area is certainly required if general trends are to be determined across multiple species.

The effective temperature for vernalization, as well as the appropriate duration at that temperature, are two important aspects of the vernalization process for a given plant species. A much more consistent flowering response was found in red sorrel seed plants and ramets that were vernalized at 4.5 ± 0.1°C than at 6°C. Effective temperatures for vernalization can range from just below or near freezing (Clarkson and Russell, 1975; Fausey and Cameron, 2007) to as high as +7 to +10°C (D’Aloia et al., 2008; Medd and Lovett, 1978; Sampson and Burrows, 1972). Temperatures between 4 and 7°C, however, are often cited as effective for many species (Boudry et al., 2002; Chouard, 1960; Foley et al., 2009; Heichel et al., 1980). The target temperature of 6°C used in the initial experiments in this study was within that range. The
variability in the response to temperature across species, however, is due to the adaptive nature of the vernalization response in a given species to different environmental conditions (Boudry et al., 2002; Kim et al., 2009). Similar variation has also been documented within different populations of the same species (Fandrich and Mallory-Smith, 2006; Fandrich et al., 2008; Roché et al., 1997) and may explain the variable response of red sorrel ramets to vernalization at 6°C. The temperature threshold for vernalization of red sorrel ramets may also be affected by pre-vernalization stimulus, as exhibited by the high flowering frequency in plants exposed to decreasing photoperiod prior to vernalization at 6°C (Table 5.17).

Duration of vernalization required to confer flowering competency in red sorrel appears to range from 10 to 15 weeks at the temperatures used in these experiments. Maximum flowering of plants established from seed occurred following the 10 week duration at 4.5 ± 0.1°C (Table 5.8), and 12 weeks at this temperature was sufficient for the successful flowering treatments in both the photoperiod X vernalization interaction and ramet removal experiments (Tables 5.5 and 5.14). The appropriate vernalization duration, much like the appropriate vernalization temperature, varies among species. However, 4 to 8 weeks of cold treatment at the appropriate temperature is often reported to be sufficient for saturating the flowering response in most species (Chouard, 1960; Fausey et al., 2006). Durations ranging from as little as 3 weeks (Schwabe, 1950) to as long as 10 to 12 weeks (Medd and Lovett, 1978; Wang et al., 2009) at the appropriate temperature are also reported. Only 10% of the seed plants exposed to 4.5 ± 0.1°C for 5 weeks flowered in our experiments (Table 5.8). Flowering competency in red sorrel therefore does not appear to be conferred by shorter durations of cold treatment at the temperature used.
It is unclear why durations of 10 and 15 weeks of vernalization at 4.5 ± 0.1°C did not saturate the flowering response in plants established from seed (Table 5.8), especially when similar durations at this temperature generally saturated the response in most of the successful treatments in the associated experiments (Tables 5.5 and 5.14). Successful vernalization, however, requires that plants be exposed to a specific series of events that ultimately culminate in a flowering response. Plants need to pass from a juvenile to responsive growth stage (Evans, 1971; Wellensiek, 1964), be hardened off or acclimated prior to exposure to cold treatment (Fausey et al., 2006; Sung and Amasino, 2004), be exposed to the proper cold temperature for the proper duration (Chouard, 1960), be exposed to an appropriate post-vernalization temperature to fix the vernalization response and prevent de-vernalization (Wang et al., 1995), and finally be exposed to the appropriate temperature and photoperiod for successful flower induction (Lang, 1952; Sung and Amasino, 2004). Absence of appropriate conditions at any of these stages can result in lack of flowering. Seed plant leaf number and ramet density were similar in all treatments prior to vernalization in the vernalization duration experiment, a trend that was similar in the majority of treatments in all experiments conducted. Flowering ramets in field populations often had less than 5 leaves “(Scott White, personal observation)” and so it seems likely that all seed plants and ramets used in the experiments conducted likely surpassed a potential juvenile growth stage prior to vernalization. It is, however, unknown if a juvenile phase exists for red sorrel prior to vernalization.

It is unclear if the 13°C and 15°C acclimation temperatures used successfully acclimated ramets and seed plants prior to vernalization or fixed the vernalized state following vernalization treatments. However, little information is available on appropriate temperatures or duration for
this process in most species as the majority of research conducted on monocarpic perennial plants omit this step. Sung and Amasino (2004) indicate that acclimation to cold temperatures generally occurs very quickly, most often within a few days of exposure to the appropriate temperature. The durations of acclimation ranged from 3 to 7 days in our experiments and should therefore have been effective if the appropriate temperatures were used. Exposure of vernalized plants to temperatures ranging from 15 to 20°C for 3 to 5 days following vernalization is often considered adequate for fixing the vernalized state in most species (Chouard, 1960; Wang et al., 1995). All vernalized ramets or seed plants in our experiments were exposed to 13°C or 15°C for 3 to 7 days following vernalization, and so it seems likely that any achieved vernalized state was successfully fixed given the available information in the literature.

While the conditions of our controlled experiments did not mimic exact field conditions in Nova Scotia, it is important to provide at least some basis for estimating the effectiveness of vernalization treatments when assessing the role of this factor in the regulation of flowering. The effectiveness of vernalization is often measured by the reduction in time (calendar days or thermal time) required for flowering (Chouard, 1960; Clough et al., 2001), the increase in the number of flowering individuals following treatment (Fausey and Cameron, 2007), or through responses such as increased plant height and flower number (Clough et al., 2001). Flowering was more or less non-existent without vernalization in our experiments, indicating an obligate vernalization requirement that was successfully met under the conditions of many of our experiments. Unfortunately the lack of flowering in unvernalized plants prevents an estimation of the reduction in time required for flowering following vernalization. A recently developed growing degree-day model for flowering of red sorrel ramets under field conditions in lowbush
blueberry (Chapter 4) does provide some basis for comparison. Initiation of flowering occurs by 285 GDD ($T_{\text{base}}=0^\circ C$) accumulating from April 1 under field conditions with 10, 50, and 90% flowering predicted to occur at 372, 544, and 880 GDD, respectively (Chapter 4). The GDD required for flowering under the controlled conditions of the vernalization experiments varied but was generally similar to the above values (Figs. 5.1, 5.3, 5.5, and 5.6) and indicates that a vernalization response similar to that which occurs under field conditions was achieved in our experiments. The onset of flowering ranged from 273 to 500 GDD in the most successful vernalization treatments across experiments with GDD to 90% flowering ranging from about 900 to 1300 GDD. The most notable exception was the delay in flowering response that was observed following vernalization at 4.5 ± 0.1$^\circ C$ for 5 weeks (Fig. 5.3). No flowering plants were observed in this treatment until 634 GDD after transfer to the grow shelves, and flowering frequency was very low (10%, Table 5.8). Flowering seed plants and ramets in this treatment had shorter flowering stems (Table 5.8) and therefore fewer flowers per stem as well (Fig. 5.2). Thus, the longer thermal time to flowering coupled with the low flowering frequency and shorter flower stem height in this treatment is strong evidence that 5 weeks of vernalization at 4.5 ± 0.1$^\circ C$ is insufficient for effective vernalization of red sorrel. Survival of varying proportions of flowering ramets was also observed at the end of many experiments (Tables 5.6, 5.9, and 5.18), similar to that observed in field populations of red sorrel ramets (Chapter 3). Although polycarpism at the ramet level has not been observed, survival of flowering ramets under both field and controlled conditions does indicate this possibility. The results of this study provide a good basis for further investigation of ramet polycarpism in red sorrel.
Pre and post-vernalization ramet removal under both field and controlled conditions successfully reduced the density of flowering ramets (Tables 5.10 and 5.14). This may provide a unique opportunity for implementing management strategies to prevent flowering and seed production in field populations of red sorrel. Field populations appear to undergo repeated seedling recruitment (Chapter 3) and therefore likely maintain high genetic diversity in established populations (Eriksson, 1989; Lei, 2010; Soane and Watkinson, 1979). High genetic diversity is considered essential to the adaptability and survival of plant populations (Booy et al., 2000). Reductions in seed production, and hence recruitment of new genets, in established red sorrel populations should therefore be a major component of any management plan. Effective management plans for monocarpic perennials or winter annuals that require vernalization include timely herbicide applications or disturbance to destroy established plants prior to vernalization or after vernalization but before flowering and seed production (Fandrich et al., 2008; Medd and Lovett, 1978; Ross and Lembi, 2009). Paraquat applications following autumn pruning or prior to spring blueberry ramet emergence significantly reduced both the net red sorrel ramet population and the density of flowering ramets within that population. Although paraquat is not a currently registered product for weed management in lowbush blueberry, other active ingredients such as dicamba are available and may provide similar reductions in both net ramet populations and density of flowering ramets with timely autumn or spring applications.

The rapid re-establishment of ramets and seed plants observed in the pre-vernalization clipping experiment indicate that red sorrel may be capable of re-establishing small ramet populations following autumn ramet removal. Similar re-establishment, however, was generally not observed following fall herbicide applications in the field experiment (Fig. 5.4). The low
flowering frequency in the pre-vernalization clipping treatment also indicates that ramets re-establishing following pre-vernalization clipping could not be induced to flower through vernalization, perhaps due to lack of adequate growth prior to vernalization. Rosette size prior to winter is a good predictor of flowering in some monocarpic perennials (Gross, 1981; Klinkhamer et al., 1991; Prins et al., 1990) as most of these plants must pass through a juvenile phase prior to vernalization to achieve flowering competency (Chouard, 1960; Evans, 1971). It is unknown if red sorrel ramets possess the same requirement, but reestablishment of large ramets was also inhibited following pre-vernalization clipping in the ramet removal experiment using plants established from root fragments (Table 5.11). Although no flowering occurred in any treatments in the experiment using ramets established from root fragments, data from the experiment using plants established from seed indicate that very young ramets re-establishing after pre-vernalization control measures likely have a low probability of flowering.

Most growers prefer to prune lowbush blueberry fields in late October or November, considerably later than the pruning date used in the ramet removal field experiment in this study. Later pruning dates may affect efficacy of fall herbicide treatments due to exposure of ramets to frost or low temperatures that may inhibit herbicide activity. Lowbush blueberry plants can be pruned as early as late September with minimal impacts on carbon assimilation (Hicklenton et al., 2000b), though repeatedly pruning fields in early Fall can decrease yields (Yarborough and Hess, 1998). Growers should therefore only consider earlier pruning dates in fields with severe red sorrel infestations to allow for timely applications of herbicides to remove overwintering ramet populations.
It should be noted that pre and post-vernalization ramet removal did not completely prevent flowering under either field or controlled conditions. This is not necessarily surprising in the field experiment as complete control of all overwintering ramets was not achieved in either the fall or the spring herbicide application treatments (Fig. 5.4). Therefore, surviving overwintering ramets contributed to flowering ramet populations in each treatment (Table 5.10). Ramets emerging in May and June also contributed to the flowering ramet populations in each treatment (Table 5.10), similar to that previously reported in field populations of red sorrel ramets (Chapter 3). The reduction in flowering obtained by ramet removal was more pronounced in the pot experiment where an average of less than 1 ramet pot\(^{-1}\) flowered following pre or post-vernalization clipping (Table 5.14). The only plant observed to flower without vernalization was found in the control treatment in this experiment, so it may be possible that some of the vernalized plants also lacked the absolute requirement for vernalization. However, the plant that flowered without vernalization required more than 100 days under a 16-hour photoperiod to flower whereas ramets re-establishing after clipping flowered within 50 days of exposure to the 16-hour photoperiod following vernalization. The few ramets that flowered in the clipping treatments, therefore, seemed to respond to vernalization in a manner similar to unclipped ramets also exposed to vernalization. Plants perceive vernalization at the site of dividing cells in meristematic tissue (Schwabe, 1954; Sheldon et al., 2000; Wellensiek, 1962), including dividing cells in root meristems (Wellensiek, 1962, 1964). It may therefore have been possible that some meristems established on the creeping root system perceived the cold stimulus during vernalization, resulting in the development of flowering ramets despite the above-ground removal of established ramets through clipping. This has not been documented in creeping perennials, however, and is merely speculation given the current level of knowledge on the
flowering biology of red sorrel. Initial attempts to vernalize root buds were also unsuccessful (data not shown).

5.6. Conclusions

The results of this study provide compelling evidence that red sorrel ramets established from creeping roots collected from established ramet populations in lowbush blueberry fields exhibit an obligate vernalization requirement to confer flowering competency. This requirement was also exhibited in plants established from seeds as less than 1% of seed plants flowered without vernalization. Vernalization at temperatures near 4°C were most effective, and vernalized ramets and seed plants required exposure to long photoperiods following vernalization for flower induction to occur. Ramets did not flower in response to increasing photoperiod without vernalization. Durations of 10 to 15 weeks at temperatures near 4°C seem to be effective for maximizing the flowering response. Flower frequency increased significantly if ramets were exposed to decreasing photoperiod prior to vernalization at 6°C, though a similar response was not found for decreasing temperature prior to vernalization. Pre and post-vernalization ramet removal can successfully reduce the density of flowering ramets and should be considered as part of an integrated management plan for red sorrel in lowbush blueberry fields.
Chapter 6: General Discussion and Conclusions

Research for this thesis involved the development of predictive models for lowbush blueberry (*Vaccinium angustifolium* Ait.) and red sorrel (*Rumex acetosella* L.) ramet emergence and development, a demographic approach to identifying key processes and structure of *R. acetosella* ramet populations in lowbush blueberry fields, and investigation into the abiotic factors regulating flowering of *R. acetosella* ramets. Predictive models, based on growing degree-days, were successfully developed for each species allowing for reliable comparisons of developmental timing of each species across seasons. The demographic approach to studying ramet dynamics and population structure of red sorrel was successful and should be incorporated into studies of other perennial weeds in lowbush blueberry. Finally, the study of vernalization and photoperiodic effects on ramet flowering of red sorrel is one of the few studies examining the role of these factors in the development of herbaceous creeping perennials. As with most research, the opportunity now exists to expand and improve upon the work completed.

6.1. Further Development and Use of Predictive Models for Lowbush Blueberry

6.1.1. Summary and Restrictions for Current Models

Growing degree-day (GDD) models were developed to predict lowbush blueberry ramet emergence and development to tip dieback in the non-bearing year and flowering in the bearing year (Chapter 2). These models are directly applicable to the major blueberry growing areas in North-Central Nova Scotia and provide the basis for expansion of this technique for modeling emergence and development of this crop in other production areas in Canada and around the world. The lowbush blueberry, however, is native to Canada and ecotypes may be locally adapted to the climate of a given production area. Temperature thresholds and response to
growing degree-days can vary among ecotypes of different weed species (Holt and Orcutt, 1996; Steinmauss et al., 2000) and has also been observed when using growing degree-days to predict harvest dates of several cultivars of highbush blueberry (*Vaccinium corymbosum*) in Michigan (Carlson and Hancock Jr., 1991). Base temperatures and degree-day thresholds established for North-Central Nova Scotia may not apply to other provinces in Canada or lowbush blueberry growing regions outside of Canada such as Maine in the United States.

6.1.2. Improving Model Performance

Model performance may be improved through experiments designed to determine accurate base temperatures for emergence, tip dieback, and flowering. Although the iterative method employed in this thesis was effective, methodology exists to determine base temperatures for sprouting of vegetative propagules and seeds (Holt and Orcutt, 1996; Steinmaus et al., 2000; Wiese and Binning, 1987). Spiers et al. (2006) have outlined a methodology for using cuttings of highbush blueberry to determine chilling requirements for bud break that could be adapted for estimating base temperatures for flowering of lowbush blueberry ramets collected from the field. Plants are also routinely established from seeds for use in pathology (Hildebrand and Braun, 1991; Hildebrand et al., 2001) and field research (Hicklenton et al., 2000a), a technique that could be extended to improving our estimates of base temperatures for rhizome sprouting and ramet development. Additional propagation methods are outlined by Morrison et al. (2000). Given the inherent variability associated with the lowbush blueberry (Glass and Percival, 2000; Hall and Aalders, 1961; Hepler and Yarborough, 1991), additional calibration and validation data sets should also be collected to improve the precision of the proposed GDD thresholds. This is particularly important for the flowering model as it was only calibrated at 2 sites. Bud burst and flowering of some clones in the bearing year is earlier than that predicted by
our flowering model (Fig. 6.1). The proportional contribution of early developing clones to the blueberry coverage of a given field will affect the applicability of the proposed flowering model. Additional work, including surveys, should be conducted to determine the contribution of these clones to overall bloom, and to assess the need to incorporate development of these clones into future predictive models. This work could be assisted by recent advances in understanding the genetic diversity of lowbush blueberry fields (Bell et al., 2008, 2009b).

![Image of two lowbush blueberry clones](image)

**Fig. 6.1.** Developmental stage of two lowbush blueberry (*Vaccinium angustifolium* Ait.) clones at the Debert Wild Blueberry Institute on May 16, 2013. The clone in panel A has nearly fully expanded leaves and several open flowers whereas leaf emergence is just beginning in the clone in panel B and no open flowers are observed. Growing degree-day (GDD, $T_{base}=0^\circ C$) accumulation for this site was approximately 270 GDD at the time photos were taken. The clone in panel B is reminiscent of the majority of clones sampled for development of the proposed growing degree-day model for predicting flowering of lowbush blueberry in Nova Scotia.

There is also opportunity to improve the model estimates of the proposed GDD thresholds by obtaining improved temperature data at field sites. Our temperature data were supplemented with early-season data from Environment Canada due to inability to establish sites early enough or get data loggers in place prior to April 1. Although accurate, Environment Canada temperature data simply was not specific to the sites at which emergence and development were monitored. Efforts should therefore be made in the future to ensure that
temperature loggers are in place at each site on the starting date for cumulative GDD calculations.

6.1.3. Role of Winter Chilling on Thermal Time to Flowering

An important consideration for the flowering model developed in this thesis is the potential effect of winter chilling on thermal time to flowering. Bud break in many woody plants growing in temperate regions is impacted by the intensity and duration of winter chilling (Cannell and Smith, 1983; Cesaraccio et al., 2004) such that adequate chilling breaks bud dormancy and enables bud break under favorable conditions (Horvath et al., 2003; Rohde and Bhalero, 2007). This can be particularly important for regulation of bud dormancy and bud break in temperate woody crop plants (Guak and Nielsen, 2013; Heide and Prestrud, 2005; Mahmood et al., 2000), including several Vaccinium species (NeSmith and Bridges, 1992; Rowland et al., 2005). Chilling requirements to break bud dormancy in lowbush blueberry are lacking, but 1000 hours of chilling at 4°C has been used to induce bud break in dormant stems used in some controlled experiments (Benoit et al., 1984; Stubbs and Drummond, 1997). Several clones studied in Maine were also reported to reach peak cold hardiness in January or February (Cappiello and Dunham, 1994). Chilling requirements for lowbush blueberry may therefore be met prior to April 1 and an earlier starting date for GDD accumulation may be appropriate in some years. Pruess (1983) has emphasized that choosing an arbitrary starting date, such as April 1, assumes negligible effects of GDD’s accumulated prior to this date. GDD ($T_{\text{base}}=0°C$) accumulations for March, however, were 69 and 40 in 2010 and 2011, respectively, according to Environment Canada temperature data at Debert, Nova Scotia. These GDD accumulations may have affected flowering time if a satisfactory chilling period had occurred prior to March 1. For example, GDD accumulations beginning on March 1 predicted flowering time to occur 7, 3, 4,
and 2 calendar days earlier in 2010, 2011, 2012, and 2013, respectively, when compared to accumulations beginning on April 1 at Debert, Nova Scotia. Variations of 2-3 days should have minimal impacts on management practices and indicate that the developed model is likely quite accurate in most years. Larger variation, however, could impact management practices such as pollination and may provide a basis for improving the ability to predict flowering. A more appropriate starting date for modeling blueberry flowering would be to begin accumulating GDD’s once the chilling requirement of the plant has been met. Chilling time can be determined under field conditions in much the same way as growing degree-days (Fraisse and Whidden, 2010) with examples for several woody plants reviewed in Cesaraccio et al. (2004). This aspect of bud burst and flowering in woody plants should be considered for future studies attempting to predict flowering of lowbush blueberry or associated woody weed species under field conditions.

6.1.4. Potential Impacts of Crop Management Practices

The effects of management practices such as pruning should also be evaluated further in terms of the effects of degree-day thresholds for emergence and development. The majority of blueberry growers prune with flail mowers in the fall, similar to the management approach for the fields used for the experiments in this thesis. GDD thresholds established in this thesis for emergence and tip dieback in the non-bearing year should therefore apply to the majority of the managed blueberry acreage in the region of model development. Spring pruning is nonetheless still a common occurrence. Timing of pruning generally has no effect on plant stand or productivity (Ismail and Yarborough, 1981), though Eaton et al. (2004) reported more rapid growth of blueberry ramets emerging in plots pruned in the fall compared to those pruned in the spring. Reasons for this are unclear but do indicate that some further work may be required to assess the impact of pruning time on blueberry ramet emergence. For example, fields pruned in
spring are unlikely to be pruned prior to April 1 and so pruning date will have to be used as the start date for GDD accumulations in spring-pruned fields. Additional work will need to be conducted to determine if a consistent GDD threshold, as suggested by Trevett (1959), exists between spring pruning date and ramet emergence in blueberry fields in Nova Scotia.

Resurgence in the use of burn-pruning for field sanitization likely warrants investigation into the effect of fall and spring burning on the thermal time to emergence. Burning tends to remove all above-ground vegetation and stimulates ramet emergence from buds on underground rhizomes; mowing only partially removes the above-ground vegetation and can stimulate emergence from buds on both rhizomes and the base of cut stems (Ismail and Hanson, 1982; Kender et al., 1964). Emergence of new ramets in mowed fields therefore occurs from vegetative structures persisting both above and below-ground. New ramets emerging from both rhizomes and the base of cut stems were counted in our study and data were combined for predicting emergence. Emergence from each of these structures, however, likely does not occur at the same GDD threshold. The majority of ramets tagged in our emergence counts emerged from rhizomes as fields were pruned quite close to soil level. Mowing as close as possible to soil level generally minimizes the number of new stems emerging from the base of cut stems (Ismail and Yarborough, 1981). New ramets emerging from the base of cut stems, however, still contribute to yield and perhaps should be modeled separately from those emerging from rhizomes.

6.1.5. Relating Pest Development to that of the Lowbush Blueberry

The ability to directly compare red sorrel phenology to that of the lowbush blueberry through the use of degree-day models has been discussed in this thesis (see Chapter 4). This ability, however, can extend to all pests in which growth and development has been modeled
using growing degree-days. *Apocynum androsaemifolium* reaches 50 and 100% emergence at approximately 184 and 420 GDD ($T_{\text{base}}=6^\circ\text{C}$), with peak flowering occurring around 750 GDD ($T_{\text{base}}=6^\circ\text{C}$) (Wu, 2010). Although emergence and development of *A. androsaemifolium* was determined on a GDD scale with $T_{\text{base}}=6^\circ\text{C}$, GDD for a particular field can be determined using multiple base temperatures. This allows for direct comparison of growth and development between blueberry ramets and the pest of interest. Gordon and Bootsma (1993) have outlined regression techniques to estimate GDD accumulation across multiple base temperatures, similar to methodology discussed by Pruess (1983). These techniques can be applied directly to any field site in which pest development is modeled using GDD’s calculated from base temperatures different from $0^\circ\text{C}$. For example, using emergence and development data for *A. androsaemifolium* (Wu, 2010) modeled using GDD ($T_{\text{base}}=6^\circ\text{C}$) a quadratic regression equation can be used to estimate the approximate GDD ($T_{\text{base}}=0^\circ\text{C}$), and thus approximate proportional blueberry emergence, tip dieback, or flowering (see Fig. 6.2). Fifty and 100% *A. androsaemifolium* emergence occurred at approximately 184 and 420 GDD ($T_{\text{base}}=6^\circ\text{C}$), respectively, which would correspond to 418 and 861 GDD ($T_{\text{base}}=0^\circ\text{C}$), respectively (Fig. 6.2). *A. androsaemifolium* therefore initiates emergence about 200 GDD later than blueberry and reaches peak emergence when blueberry ramets are between 90 and 95% emergence (Fig. 2.2). Peak flowering of *A. androsaemifolium* occurred at 750 GDD ($T_{\text{base}}=6^\circ\text{C}$) (Wu, 2010), which would correspond to 1366 GDD ($T_{\text{base}}=0^\circ\text{C}$). *A. androsaemifolium* therefore flowers much later than blueberry and may serve as a source of late-season nectar or pollen for native pollinators in lowbush blueberry fields. Ramets of *A. androsaemifolium*, however, are much taller than blueberry ramets and therefore inhibit harvest operations in bearing-year fields. *A. androsaemifolium* is most susceptible to herbicides at the early flowering stage and early in the
fall before the first killing frost (Wu, 2010); the later flowering time of *A. androsaemifolium* likely prevents satisfactory control and desiccation of stems prior to harvest. Using a similar approach, approximate GDD thresholds for a variety of base temperatures can be related to estimated proportional blueberry emergence and development (Table 6.1). As GDD estimates for blueberry emergence and development continue to improve these types of comparisons should become much more accurate.
Fig. 6.2. Regression of cumulative growing degree-days calculated at a base temperature of 0°C on cumulative growing degree-days calculated at a base temperature of 6°C at the Purdy research site in Collingwood, Nova Scotia in 2010. The fitted regression equation is a quadratic equation of the form $y = a + bx + cx^2$. Parameter estimates were obtained from the regression wizard function in SigmaPlot® version 12.0.
Table 6.1. GDD thresholds for proportional cumulative lowbush blueberry ramet emergence and development and corresponding GDD thresholds at alternative base temperatures determined by linear and quadratic regression of cumulative GDD ($T_{\text{base}}=0^\circ\text{C}$) against cumulative GDD calculated using the indicated base temperatures.

<table>
<thead>
<tr>
<th>Blueberry Growth Stage</th>
<th>Proportion of Ramet Population</th>
<th>GDD Threshold ($T_{\text{base}}=0^\circ\text{C}$)</th>
<th>Approximate GDD Thresholds for Common Base Temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$T_{\text{base}}=1^\circ\text{C}^x$ $T_{\text{base}}=2^\circ\text{C}^x$ $T_{\text{base}}=3^\circ\text{C}^x$ $T_{\text{base}}=4^\circ\text{C}^x$ $T_{\text{base}}=5^\circ\text{C}^w$ $T_{\text{base}}=6^\circ\text{C}^w$</td>
</tr>
<tr>
<td>Percent Cumulative Ramet Emergence</td>
<td>10</td>
<td>276</td>
<td>242 210 182 154 140 117</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>407</td>
<td>367 327 290 253 214 180</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>734</td>
<td>670 609 551 494 407 350</td>
</tr>
<tr>
<td>Percent Cumulative Ramets at Tip Dieback</td>
<td>10</td>
<td>834</td>
<td>766 698 634 570 473 409</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>1082</td>
<td>994 911 831 751 639 560</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1473</td>
<td>1363 1255 1149 1044 932 832</td>
</tr>
<tr>
<td>Percent Cumulative Flowering Ramets</td>
<td>10</td>
<td>410</td>
<td>367 327 292 253 214 180</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>477</td>
<td>433 389 346 306 254 215</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>595</td>
<td>537 485 437 390 319 272</td>
</tr>
<tr>
<td>Peak Bloom</td>
<td></td>
<td>559$^y$</td>
<td>507 458 411 365 300 256</td>
</tr>
</tbody>
</table>

$^x$White et al. 2012 and Chapter 2.
$^y$Mean of estimated GDD for peak bloom from two sites taken from White et al. 2012.
$^x$GDD estimates from linear regression of cumulative GDD ($T_{\text{base}}=0^\circ\text{C}$) against cumulative GDD at indicated base temperature.
$^w$GDD estimates from quadratic regression of cumulative GDD ($T_{\text{base}}=0^\circ\text{C}$) against cumulative GDD at indicated base temperature.
6.2. Role of Demography for Management and Study of Perennial Weeds in Lowbush Blueberry Fields

Herbaceous creeping perennials inhabiting lowbush blueberry fields are not disrupted by traditional agronomic practices such as tillage and crop rotation. Limited herbicide options for management of herbaceous creeping perennials such as *R. acetosella* therefore results in proliferation of these species in an agroecosystem oriented towards promoting the growth of a perennial crop. Under such conditions, effective weed management requires knowledge of population-level processes that can most effectively be explored through demographic methods. In the context of weed science, Ghersa et al. (2000) indicate that demographics identify processes critical to weed population growth and allow for design of management strategies that have a high impact on these processes. This thesis provides the first attempt at implementing a demographic approach to studying perennial weeds in lowbush blueberry fields. Results reported herein have identified some of the key processes regulating populations of *R. acetosella* in lowbush blueberry, and the proposed life-cycle model can serve as a tool to help predict the impacts of management practices on these key processes and overall population dynamics of *R. acetosella*.

6.2.1. Key Processes Regulating *Rumex acetosella* Populations and the Role of the Proposed Life-Cycle Model for Developing Management Strategies

Research for this thesis highlighted three main components of *R. acetosella* populations in lowbush blueberry fields in Nova Scotia; the initial overwintering ramet population, the population of new ramets emerging within each season, and seedlings (Fig. 3.8). The majority of the flowering population is comprised of ramets from the overwintering cohort (Tables 3.6 and 3.7), and surviving vegetative and flowering ramets from this cohort (Figs. 3.5 and 3.6)
contribute to final net ramet populations at the end of each season (Figs. 3.7 and 3.8). A vernalization requirement for flowering was confirmed (Chapter 5), and this is the first report of the mechanism regulating developmental segregation of ramets for this species. Exposure of ramets to the cold temperatures of winter is therefore a key process in the reproductive biology of *R. acetosella*. Identification of this process was possible primarily through the detailed tagging and subsequent monitoring of ramet phenological development incorporated into the demographic approach used. Based on the proposed life-cycle model (Fig. 3.8), management strategies designed to reduce or eliminate overwintering ramets should reduce or prevent flowering. Initiation of a management strategy should therefore begin after fall pruning at the end of the bearing year when there is an opportunity to remove net ramet populations using non-selective herbicides with minimal risk of injury to blueberry plants. This could also be effective in early spring of the non-bearing year provided herbicides are applied prior to 242 GDD (*T*\(_{\text{base}}\)=0°C; Fig. 2.2). Initial attempts at this strategy indicate large reductions in flowering ramet density under both controlled and field conditions (Tables 5.13 and 5.17). Future research should investigate the efficacy of alternative herbicide products or burning for removing overwintering ramets, and also investigate the impact of ramet removal on seed production and seedbank dynamics. The demographic approach could also be used to identify similar flowering behavior in other creeping perennials (e.g. *Heiracium* spp.) in lowbush blueberry and aid in the development of similar management strategies.

In this study it was found that new *R. acetosella* ramets emerged throughout each season (Figs. 3.1 and 3.2) with multiple cohorts of new ramets contributing to final net ramet populations at each site (Fig. 3.7). The majority of these new ramets (>90%) remained vegetative
in the year of emergence (Tables 3.6 and 3.7; Fig. 3.8). Ramets emerging in a given season therefore contribute to the persistence of established genets while simultaneously contributing to flowering and seed production in the following season. Season-long emergence of vegetative ramets is therefore a key process contributing to the persistence and spread of *R. acetosella* in lowbush blueberry fields. Season-long emergence could have been determined without detailed tracking of ramets, but the lack of flowering in most new ramets (Tables 3.6 and 3.7; Fig. 3.8) would have been difficult to elucidate without the demographic methods used. The order imposed on a plant population through demographic methods is therefore crucial to identify even simple patterns in developmental segregation. Based on the proposed life-cycle model (Fig. 3.8), management strategies to prevent or inhibit season-long emergence of vegetative ramets will be important for reducing final net ramet populations at the end of a given season. Following removal of net ramet populations at the end of the bearing year, suppression of vegetative ramet emergence in the non-bearing year will minimize the size of the net ramet population surviving into the subsequent bearing year (Fig. 3.8). This should reduce the density of flowering ramets in the bearing year and minimize the potential impacts of the overlapping flowering period of these species (see Chapter 4). Effective strategies for suppressing season-long ramet emergence, however, are limited in lowbush blueberry. New *R. acetosella* ramets stay as vegetative rosettes in the year of emergence and therefore remain protected below the blueberry canopy. Growers therefore rely heavily on preemergence residual herbicides for suppression of *R. acetosella*. Control from currently registered products, however, is variable. Future research should focus on new herbicides with similar use patterns to those already registered to inhibit season-long emergence of vegetative ramets.
Seasonal recruitment of new seedlings into established *R. acetosella* populations occurred at all sites monitored for this thesis. Seedlings generally emerged throughout the season (Fig. 3.3) with seedling survival averaging 19 ± 4% across sites (Fig. 3.8). These data indicate that there may be substantial seasonal incorporation of new genets into established populations, a key process in the maintenance of genetic diversity of perennial plant populations (Eriksson, 1989) and the first report of this in established red sorrel populations. The actual long-term survival and successful establishment of surviving seedlings from a given season, however, is still unknown. Seedling emergence was monitored at most sites for only one season and therefore long-term survival and actual recruitment of these seedlings into established populations was not determined for this thesis. Genet dynamics of clonal plants are complex (Eriksson, 1989, 1993) and seedlings of many perennial species are subject to mortality for multiple years following emergence (Antonovics and Primack, 1982; Bishop et al., 1978; Hartnett and Bazzaz, 1985a; Sarukhan and Harper, 1973). Experiments to monitor long-term survival and dynamics of *R. acetosella* seedlings are therefore required to accurately determine the probability of an emerged seedling surviving to become an established, ramet-producing genet in a lowbush blueberry field. This could also be useful for improving predictions of genet recruitment in the life-cycle model (Fig. 3.8). Data from this thesis nonetheless provide evidence that prevention of seedling recruitment should be a component of a comprehensive management strategy for *R. acetosella* in lowbush blueberry. Use of fall or spring herbicide applications to remove overwintering ramets (described above) should also be effective for killing newly established seedlings, but this has not been evaluated. Similarly, strategies to suppress the season-long emergence of vegetative ramets in the non-bearing year will likely suppress seedling establishment during this period.
Future research on new herbicides should incorporate data collection on both ramet and seedling mortality to ensure prevention of seedling establishment by new herbicides under consideration.

Finally, the new knowledge of *R. acetosella* population dynamics and the availability of the life-cycle model for predicting dynamics of this species in lowbush blueberry fields in Nova Scotia should be useful for improving future research investigating the effects of management practices on this species. For example, Kennedy et al. (2011) investigated the effects of hexazinone and fertilizer on vegetative growth and seed production of red sorrel ramets in non-bearing year lowbush blueberry fields in Nova Scotia. Applications of up to 40 kg N ha\(^{-1}\) did not significantly affect reproductive biomass or seed weight, and only increased seed production at one of the four sites used in the study. Effects of fertilizer applications, however, were measured in the year of application during the non-bearing year (Kennedy et al., 2011). The measurements of flower biomass and seed production were therefore likely taken on ramets that established in the previous bearing year but overwintered and flowered in the year that the experiment was established. The true effects of the fertilizer applications on flowering and seed production would likely be more apparent in the vegetative ramets establishing in the year of fertilizer applications but overwintering and flowering in the subsequent year. Our new knowledge on the biology of *R. acetosella* thus allows for development of new hypotheses regarding the downstream effects of management practices, such as fertilizer applications, on *R. acetosella* ramet development. The transition probabilities in the life-cycle model also allow for tests of the impacts of these management practices on deviations in predicted survival and proportional distribution of flowering and vegetative ramets. For example, a quadrat with 100 ramets alive at the end of the bearing year should have 88 living ramets in the following spring, of which 60 will flower and
28 will remain vegetative. Approximately 34 and 10 of the flowering and vegetative ramets, respectively, should survive and contribute to the net ramet population in that quadrat at the end of the non-bearing year. Do fertilizer applications affect this proportional distribution of vegetative and flowering ramets? Would this also affect winter survival? A basis for exploring these questions has been established in this thesis and the effectiveness of the life-cycle model, in this respect, will improve through continued refinement.

6.2.2. Improving the Proposed Life-Cycle Model for *Rumex acetosella*

The transition probabilities that predict the outcome of the life-cycle model are means of observed field data, the precision of which can only be improved through similar research conducted at additional field sites. In particular, the survival probabilities of new and overwintering ramets in the non-bearing year is only based on data from Pigeon Hill-2010 as elastic color of dead ramets was not recorded in the non-bearing years at the Wyvern and Purdy sites. The non-bearing year trends in ramet mortality were similar at the Wyvern and Purdy sites (Figs. 3.1 and 3.2), but actual transition probabilities are required for the model. Availability of the current life-cycle model, however, provides an important framework that can serve as a guide for additional data collection by allowing future researchers to focus on specific processes regulating ramet populations.

Survival of new vegetative ramets in the bearing year varied significantly across sites (see page 88), indicating that a single mean transition probability for these ramets may not be appropriate for predictive purposes. Additional data to improve our estimates of these probabilities is therefore important for effective predictive use of the life-cycle model. Seedling emergence and survival data for the life-cycle model was also determined primarily at the
bearing year sites. Ramet dynamics varied between the non-bearing and bearing years (Figs. 3.1 and 3.2), but it is unclear if seedling dynamics follow a similar trend. The current life-cycle model is designed to predict both non-bearing and bearing year seedling recruitment. Seedling data specific to the non-bearing year should therefore be collected to determine if adjustments to the life-cycle model are necessary.

The transition probabilities used for the life-cycle model predict ramet survival and the transition of ramets from a vegetative to flowering life history stage, similar to that used in other demographic studies of ramets (Bishop et al., 1978; Navas and Garnier, 1990). The probability of ramet survival or flowering, however, is often affected by ramet size or age (Eriksson, 1988; Nault and Gagnon, 1993; Pitelka et al., 1985) and a more detailed life-cycle model for *R. acetosella* may therefore be possible. Future research should investigate the possibility of additional ramet life-history stages for the model, likely based on ramet size (e.g. leaf number) or age. Ramet mortality was routinely observed in plants established from seed for the flowering experiments described in Chapter 5. Preliminary work under controlled conditions could therefore be conducted to investigate the potential role of ramet size or age on survival and to develop an appropriate size-classification scheme for *R. acetosella* ramets. The developed classification scheme could then be extended to field populations of ramets through additional research. A similar approach could also be used to improve our ability to predict the proportion of flowering ramets if flowering is affected by ramet size or age (see juvenility discussion below).
Estimates of seed production could also be incorporated into the model. Estimates of ramet sex ratios are provided (Table 3.9) that could be incorporated into the model to predict the proportion of male and female ramets in flowering ramet populations. Current estimates of seed production per ramet (Kennedy et al., 2011) could then be combined with the estimated proportion of female ramets to predict seed production in a given year based solely on the number of overwintering ramets. A 1:1 ratio of male and female ramets was observed at most of the study sites (Table 3.9), indicating that half of all flowering ramets could be expected to be female. Sex ratio data for this thesis was limited to flowering ramets occurring within established quadrats, but it is the best estimate of sex ratios for this species available in lowbush blueberry. Methods are described for estimating sex ratios of this species over larger areas (Putwain and Harper, 1972) and could be used to more accurately estimate sex ratios of *R. acetosella* populations at the field scale to improve model predictions.

6.2.3. Role of the Proposed Life-cycle Model for Managing Herbicide Resistant *Rumex acetosella* Populations in Lowbush Blueberry

Lowbush blueberry growers rely heavily on the use of the preemergence herbicide hexazinone for managing weeds. Exclusive reliance on this herbicide, however, selects for weeds naturally tolerant to the herbicide (Yarborough and Bhowmik, 1989) or leads to the development of hexazinone resistance (Burgess, 2002; Jensen and Yarborough, 2004). Control of *R. acetosella* from preemergence applications of hexazinone is currently highly variable (Kennedy et al., 2010, 2011) and hexazinone resistant biotypes have recently been identified in many fields (Li, 2013). A variety of management practices can be used to manage herbicide resistance in most agricultural production systems (Vencill et al., 2012), with annual rotation of herbicide modes of action providing one of the most feasible strategies for lowbush blueberry. The primary difficulty
with implementing this strategy is the identification of appropriate application timings for the available herbicide modes of action. The proposed life-cycle model for *R. acetosella* provides a detailed blueprint of the growth and development of this species over the two-year blueberry production cycle and can be used to identify application timings for various herbicides registered for lowbush blueberry. Given the current list of registered herbicides for lowbush blueberry in Canada (Anonymous, 2012), application timings for potentially effective herbicide modes of action are provided (Fig. 6.3) that can be used as guide to develop an annual herbicide rotation. Enough herbicide modes of action are currently available to manage *R. acetosella*; the life-cycle model simply provides the framework for identifying the most effective application timings and developing the herbicide rotation.

![Life-cycle model diagram](image)

**Fig. 6.3.** Use of the proposed life-cycle model for *Rumex acetosella* L., modified from Chapter 3, to develop an herbicide rotation for resistance management in lowbush blueberry.

### 6.2.4. Use of Demographic Data to Study Carbohydrate Movement in *Rumex acetosella*

The segregation of flowering and vegetative ramets in *R. acetosella* also provides the opportunity to explore hypotheses regarding the physiological integration between ramets and
carbohydrate movement within genets as this can aid in the development of effective management strategies. A balance between vegetative and flowering ramets is essential for growth of genets of clonal plants as flowering ramets seldom export assimilates to support growth of the genet in many species (Noble and Marshall, 1983; Ong et al., 1978; Pitelka and Ashmun, 1985). Similar patterns of assimilate movement, however, have not been documented in *R. acetosella* but could be explored under field and controlled conditions now that the requirements for flowering have been confirmed. It is also unclear which portion of the ramet population contributes assimilates to growth of the creeping root system, or what the patterns of assimilate movement are in this species. Ramets depend on resources from the genet during initial phases of growth (Bullock et al., 1994; Hartnett and Bazzaz, 1983) but ultimately become independent and return resources to the genet (Bradbury and Hofstra, 1977; D’Hertefeldt and Jónsdóttir, 1999). This behavior results in predictable movement of carbohydrates from creeping roots to young ramets and subsequently back to roots in creeping perennial weeds such as *Apocynum cannabinum*, *Cirsium arvense* and *Convolvulus arvensis* L. (Becker and Fawcett, 1998; Frazier, 1943; McAllister and Haderlie, 1985). This predictability can be exploited by timely management practices designed to either remove emerged ramets when root reserves are low or facilitate herbicide movement with assimilates from ramets back down to depleted root tissues. Species such as *Apocynum cannabinum* and *Cirsium arvense*, however, have distinct peaks in ramet emergence (Donald, 2000; Webster and Cardina, 1999; Wu, 2010) and produce ramets that flower and senesce in the year of emergence (Moore, 1975; Robison and Jeffrey, 1972). Ramet dynamics of *R. acetosella* are more complex and season-long emergence of vegetative ramets likely indicates a constant dynamic of upward and downward movement of carbohydrates between the root system and ramets. This likely prevents the occurrence of
predictable seasonal depletions of root carbohydrates and may limit the applicability of management strategies designed to deplete root reserves.

6.3. Prospects of Studying Vernalization in an Herbaceous Creeping Perennial

*Rumex acetosella* ramets established from creeping roots collected from established field populations had an obligate vernalization requirement for flowering. This requirement was also found in seed plants as less than 1% of plants established from seed flowered during the experiments conducted. The role of vernalization in flowering competency of herbaceous perennial plants has primarily been studied in monocot species (Davy, 1982; Hodgkinson and Quinn, 1978; McCown and Peterson, 1964; Noble et al., 1979; Wycherly, 1954) and is rarely studied in broadleaf herbaceous creeping perennials. This thesis represents the first research into the effects of vernalization on ramet development in *R. acetosella*. The identification of this response in *R. acetosella* provides a potential model species for studying the role of vernalization on genet and ramet development in broadleaf herbaceous creeping perennials.

6.3.1. Impacts of Vernalization on Seeds and Root Buds

Vernalization is the acquisition or acceleration of the ability of plants to flower following exposure to cold temperatures (Chouard, 1960). Imbibed and germinating seeds (Nordborg and Bergelson, 1999; Prince and Marks, 1982), plant apical meristems (Schwabe, 1954), leaf cuttings (Wellensiek, 1964), and root cuttings (Wellensiek, 1962) have all proven responsive to vernalization. Vernalization of imbibed or germinating seeds has been documented in several monocot species (Donald, 1984; McCown and Peterson, 1964; Sampson and Burrows, 1972; Wang et al., 1995). Dicot species that require vernalization, however, are often classified as species with imbibed seed or germinating seeds sensitive to vernalization (Clarkson and Russel,
1975; Nordborg and Bergelson, 1999; Prince and Marks, 1982; Prince et al., 1978) and those that possess a juvenile phase that must be surpassed prior to vernalization (Baskin and Baskin, 1979; Cuthbertson, 1965). Research into the effects of vernalization on imbibed or germinating seeds of broadleaf herbaceous creeping perennials has not been conducted. *R. acetosella* seedlings remain vegetative in the year of emergence under field conditions (Chapter 3), indicating that vernalization of imbibed seeds or young seedlings does not occur under field conditions in lowbush blueberry. Imbibed or germinating seeds, however, are likely not exposed to sufficient duration of cold during most of the emergence period observed in lowbush blueberry fields (Fig. 3.3). Lack of seedling flowering therefore does not necessarily indicate lack of responsiveness of germinating seeds or young seedlings to vernalization. Emergence of new seedlings was observed throughout the fall at many of the study sites as well (Fig. 3.3). Imbibed seeds or young seedlings emerging late in the season would be exposed to the appropriate duration of cold to potentially flower in the following season. Winter survival of young seedlings, however, is still unclear. It is also unknown if young seedlings of *R. acetosella* exhibit juvenile stages that lack responsiveness to environmental stimuli, such as vernalization.

Vernalized root cuttings of *Lunaria biennis* were found to regenerate into flowering plants when exposed to sufficient duration of cold exposure at 5°C (Wellensiek, 1962). Similar results have been reported with root tissues of *Cichorium intybus* L. (Pierik, 1966) and *Arabidopsis thaliana* (Burn et al., 1993) and indicate that vernalization of adventitious root buds in *R. acetosella* may potentially culminate in the emergence of flowering ramets. Initial attempts to explore this possibility for this thesis were unsuccessful, primarily due to sprouting and growth of ramets from root fragments planted and stored at 4.5 ± 0.1°C. Creeping roots of some
perennial species continue to grow almost year-round as long as temperature permits sprouting and growth of root buds (Donald, 2000; Liew et al., 2012; McAllister and Haderlie, 1985). Root buds of *Cirsium arvense*, for example, grow towards the soil surface during the winter prior to emergence in the spring (Donald, 2000; McAllister and Haderlie, 1985). Slow growth and cell division in root buds of *R. acetosella* under the cold soil conditions of winter might therefore be sufficient to result in vernalization of the dividing cells and emergence of a flowering ramet. *R. acetosella* roots sprout at low temperatures (Figs. 4.2 and 4.3) and ramets emerge from early spring until late fall or early winter under field conditions (Figs. 3.1, 3.2 and 4.4). The possibility of vernalization of root buds should therefore be explored further to determine if this can contribute to the reproductive biology of this species under field conditions in Nova Scotia.

6.3.2. Impacts of Juvenility on Vernalization of *Rumex acetosella*

Many plant species that require vernalization for flowering competency exist on a continuum of increasing sensitivity to vernalization with increasing plant age (Klinkhamer et al., 1987b). Early stages of growth in which young plants are non-responsive to vernalization are referred to as juvenile stages (Fausey et al., 2006; Wellensiek, 1964). Juvenility must be determined on a species by species basis (Chouard, 1960) and data on juvenility of ramets or genets of clonal plants is limited (Sachs, 2002). A juvenile stage exists in tillers of some perennial grasses (Foggo and Warrington, 1989) and ramets of *Carex arenaria* (Noble and Marshall, 1983) and therefore may also exist in ramets of *R. acetosella*. Ramets and seed plants used in our experiments were generally grown for 2-4 months prior to vernalization with leaf number ranging from 6 ± 1.2 to 20.3 ± 3.5 for ramets and 49.1 ± 2 to 153 ± 13 for plants established from seed. The juvenile stage for many rosette-forming vegetable crops ranges from 2 to 15 leaves (Atherton et al., 1990; Weibe, 1990), similar to that reported for the monocarpic
perennial *Centaurea diffusa* (Thompson and Stout, 1991). The model perennial species *Arabis alpina* must be at least 5 weeks of age to respond to vernalization when established from seed (Wang et al., 2011). Ramets and seed plants used in the experiments in this thesis therefore likely exceeded any potential juvenile stage associated with *R. acetosella*. The existence of a juvenile stage for this species, however, is unknown and has both practical and biological implications for future research with this species. From a practical view, identifying the duration of a potential juvenile phase could drastically reduce the duration of vernalization experiments with this species by reducing the amount of time that plants are grown prior to vernalization. For example, overwintering ramets were observed to flower with fewer than 5 leaves under field conditions (Fig. 6.4) which may indicate sensitivity of younger ramets to vernalization than those used in the experiments in Chapter 5. The juvenile phase could also be incorporated into the proposed life-cycle model for ramet field populations (Fig. 3.8). From a biological view, the potential for a juvenile phase in *R. acetosella* exists at both the genet and ramet level and poses interesting opportunities for the study of this phenomenon in creeping perennials. At the genet level, juvenility in an establishing seedling may persist to ramets emerging from creeping roots produced by that seedling. For example, vernalization of leaf cuttings from juvenile plants of *Lunaria biennis*, a vernalization requiring monocarpic perennial, do not flower whereas the same procedure using leaves taken from adult plants yields flowering plants (Wellensiek, 1961). The ability of dividing cells in root tissue or imbibed seeds to be vernalized and yield flowering plants also requires absence of a juvenile stage (Chouard, 1960; Heide, 1994; Pierik, 1966). The existence of a juvenile phase in *A. alpina* (Wang et al., 2011) may be indicative of other perennial plants and eliminate the possibility of vernalizing seeds or root buds. Identification of
juvenility in *R. acetosella* will therefore be important for future research into the effects of vernalization on flowering.

![Image](image.png)

Fig. 6.4. Overwintering ramet of red sorrel (*Rumex acetosella* L.) bolting in early May in a lowbush blueberry (*Vaccinium angustifolium* Ait.) field in Collingwood, Nova Scotia.

6.3.3. Timing of Flower Primordia Formation

The proportion of overwintering ramets flowering under field conditions ranged from 39-75% (Chapter 3), similar to proportions of vernalized ramets flowering under controlled conditions (Tables 5.5, 5.14 and 5.17). The vernalization response obtained under controlled conditions was therefore very similar to that observed under field conditions. Reasons for lack of flowering in all ramets exposed to cold temperatures, however, are currently unclear. Chouard (1960) indicated that vernalization does not directly induce the formation of flower primordia, but rather creates the capacity for subsequent flowering in vernalized plants exposed to inductive conditions after chilling. Recent research with the perennial plant *Arabis alpina*, however, has found that only apical meristems of the main and axillary shoots that actually initiate flower primordia during the cold treatment produce flowers when returned to warm temperatures.
(Amasino, 2009; Wang et al., 2009; Wang et al., 2011). Formation of flower primordia during chilling was also restricted to meristems of the main and axillary shoots that were formed prior to vernalization (Wang et al., 2009). Apical meristems were not inspected microscopically for flower primordia during our research, so it is currently unclear if *R. acetosella* undergoes a similar pattern of floral initiation during vernalization. Contrary to results of Wang et al. (2009), however, is the fact that many *R. acetosella* ramets formed prior to vernalization failed to flower when returned to inductive conditions under both field and controlled conditions. Rosettes of the monocarpic perennial Hound’s tongue (*Cynoglossum officinale* L.) also form flower primordia during vernalization (De Jong et al., 1986), but only in rosettes that reach a critical size prior to the onset of vernalizing temperatures. Rosettes below the critical size do not initiate flower primordia during vernalization and remain vegetative when returned to warm temperatures and inductive photoperiods (De Jong et al., 1986). In this thesis it was shown that field populations of *R. acetosella* ramets obtain a distinct age structure at the end of each season (Fig. 3.7) and thus contain ramets of different calendar ages that may vary in their responsiveness to vernalization. The implications of juvenility on the flowering response of *R. acetosella* have been discussed above but may also be helpful in explaining the lack of flowering in all ramets exposed to cold temperatures. Finally, understanding the timing of flower primordia development in response to vernalization may also help improve our understanding of the stability of the vernalized state in ramets of *R. acetosella*. Stability of the vernalized state is conferred through stability of the repression of floral inhibiting genes after vernalized plants are returned to warm temperatures (D’Aloia and Périlleux, 2008; Shindo et al., 2006). This repression is mitotically stable (Sung and Amasino, 2004) and vernalized plants can retain the ability to flower for prolonged periods following vernalization (Amasino, 2010). This stability is
variable (Shindo et al., 2006) or lacking (Wang et al., 2009), but has not been extensively studied in ramets of herbaceous creeping perennials.

6.3.4. Identification of the Optimum Temperature for Vernalization

Although the vernalization requirement of R. acetosella has been confirmed, the exact interaction between vernalization temperature and duration in R. acetosella is yet to be determined. Temperatures between 4 and 7°C are often cited as effective for vernalizing many species (Boudry et al., 2002; Chouard, 1960; Foley et al., 2009), though effective temperatures can range from just below or near freezing (Clarkson and Russell, 1975; Fausey and Cameron, 2007; Hansel, 1953) to as high as +7 to +10°C (Medd and Lovett, 1978; Sampson and Burrows, 1972). Temperatures near 4°C were more effective for vernalizing R. acetosella than 6°C in our experiments, but lower temperatures could not be investigated. An effective temperature for vernalization was therefore identified, but the optimum vernalization temperature for this species remains unknown. Durations of at least 10 weeks at 4.5 ± 0.1°C were required to induce flowering competency in our experiments, but this duration may be shorter at cooler temperatures. For example, duration of cold treatment to saturate the flowering response of the ornamental plant Veronica spicata decreased from 32 to 20 days when the vernalization temperature was decreased from 2.5 to -2.5°C, respectively (Fausey and Cameron, 2007). There is also opportunity to explore this further in field populations as well by monitoring cooling days (Davis and Raghu, 2010, see above blueberry discussion as well) throughout the fall and collecting plants to monitor for flowering under inductive conditions. This could be used to predict the onset of the vernalized state and provide a basis for determining timing of flower primordia development under field conditions.
6.3.5. Identification of the Optimum Photoperiod for Flowering

It has been established that *R. acetosella* requires vernalization followed by exposure to long days to flower (Chapter 5). Long-day plants are those in which flowers are only formed in photoperiods that exceed a critical length (Lang, 1952). The experimental design used to test the effect of photoperiod and vernalization on flowering (Table 5.5), however, was designed primarily to determine if *R. acetosella* ramets flowered in response to increasing photoperiod. The experiment successfully showed that ramets do not flower in response to increasing photoperiod and that cold exposure followed by long days is required for flowering (Table 5.5). However, the experiment did not specifically address the impact of post-vernalization photoperiod on flowering. For example, ramets maintained under the pre-vernalization 16 hour photoperiod were not transferred to both 8 and 16 hour photoperiods following vernalization. A simpler experiment in which ramets are grown under constant photoperiod and then transferred to various photoperiods following vernalization should be conducted to identify the critical daylength for flowering. Timing of flowering in field populations of *R. acetosella* in relation to daylength varies across regions and ecotypes (Harris, 1970), similar to that reported for some other *Rumex* species (Hume and Cavers, 1983). Identification of a critical day length for flowering in Nova Scotia would therefore provide some basis for comparison to the flowering time of this species in other regions. Ramets were observed to begin bolting under field conditions in early May when day length in Nova Scotia is approximately 14 hours. The critical photoperiod for flower induction is therefore likely somewhat shorter than the 16 hour photoperiod used for the long day treatment in our experiments.
6.3.6. Movement of the Floral Stimulus in *Rumex acetosella*

The identification of a vernalization requirement in *R. acetosella* may provide the opportunity for studying the movement of the floral stimulus between ramets of a broadleaf herbaceous creeping perennial. *R. acetosella* ramets emerging in May and June under field conditions contributed to flowering ramet populations at most field sites (Tables 3.6 and 3.7), despite lack of direct exposure to vernalization. Although the vernalization requirement may not exist for all *R. acetosella* genotypes in lowbush blueberry fields, the results of experiments in Chapter 5 certainly support a vernalization requirement for ramet flowering in this species in Nova Scotia. Flowering of new ramets emerging in early spring may therefore be the result of the translocation of a mobile floral stimulus from vernalized to unvernalized ramets. The transmission of a flowering induction stimulus has been observed in tillers of some temperate grasses following induction of flowering competency by short days (Havstad et al., 2004). These authors reported that anywhere from 15 to 27% of tillers formed following transfer of plants from short to long days became reproductive, despite lack of exposure of these tillers to the short days required to confer flower competency. The results indicate movement of a mobile floral stimulus (Havstad et al., 2004) that may also occur between ramets of *R. acetosella*. A mobile floral stimulus in plants has recently been identified in the model plant *Arabidopsis thaliana* (Jaeger and Wigge, 2007). The stimulus is a protein synthesised by messenger RNA from a gene called *FT* in response to inductive photoperiods (Jaeger and Wigge, 2007) and is likely the long sought mobile floral stimulus “florigen” (Zeevaart, 2008). Current models on the movement of the *FT* protein depict movement from a leaf to a shoot apical meristem located above the leaf (Amasino, 2010; Turck et al., 2008; Zeevaart, 2008). Results of Havstad et al. (2004), however, indicate downward movement of this stimulus through the phloem from one tiller to another.
Movement of the stimulus does not occur in all grass species studied (Lindsey and Peterson, 1964) and it is unclear if similar movement of a mobile floral stimulus would occur between ramets of *R. acetosella*. Movement of nutrients between *R. acetosella* ramets is thought to be limited (Klimeš and Klimišová, 1999), but movement of photoassimilates between ramets does occur (Harris, 1972). This indicates a certain level of physiological integration between ramets. Although purely speculation, physiological integration may allow for movement of a phloem-mobile floral stimulus between ramets and potentially impact the reproductive biology of this species in lowbush blueberry fields.

6.3.7. Vernalization-Induced Genetic Changes in *Rumex acetosella*

*Rumex acetosella* may provide an interesting opportunity to test hypotheses regarding the genetic changes associated with vernalization in herbaceous creeping perennials. As discussed above, the timing of flower initiation in response to cold temperatures will be an important component of the genetic response to vernalization in *R. acetosella*. Maintenance of vegetative meristems in *A. alpina* is due to a transient repression of the flower-inhibiting gene *PEP1* by vernalization; transfer of vernalized plants back to warm temperatures resulted in renewed upregulation of *PEP1* and thus the repression of flowering in meristems that had not already initiated flower primorida (Wang et al., 2009). A similar molecular response may occur in *R. acetosella* that contributes to the maintenance of both vegetative and flowering ramets. Stable suppression of flower inhibiting genes, however, has been observed in vernalized tillers of the perennial grass *Phleum pretense* (Seppänen et al., 2010). Stable suppression of these genes, and thus flowering, does not occur in unvernalized tillers (Seppänen et al., 2010). Flowering in timothy genets is thus partly regulated by genetic changes at the tiller level of organization. Similar genetic regulation of flowering may also occur at the ramet level of organization in *R.*
acetosella and could potentially be discerned through detection or lack of detection of certain genes in flowering versus vegetative ramets. Genetic changes associated with vernalization in monocarpic perennials results in an irreversible transition to flowering and ultimately death of the individual plant, or genet. Confinement of these genetic changes to a certain proportion of ramets or apical meristems in polycarpic perennials isolates senescence and results in maintenance of the genet across multiple seasons. Wang et al. (2009) provide a basis for comparison of the vernalization response in polycarpic herbaceous perennials. Identification of additional study species, such as R. acetosella, provides the opportunity for further exploration of the role of vernalization in maintaining polycarpy in these plants.

6.3.8. Understanding the Role of Pre-vernalization Stimuli on Vernalization and Flowering Competency

The role of pre-vernalization stimuli on the flowering response of R. acetosella requires further study as this factor appears to play an important role in the flowering biology of this species in lowbush blueberry fields in Nova Scotia. Flowering frequency was reduced in ramets maintained under 8 hour photoperiods prior to vernalization when compared to those maintained under 16 hour photoperiod (Table 5.5). Increased flowering frequency was also observed in ramets exposed to decreasing photoperiods prior to vernalization (Table 5.17), the first report of this in ramets of a herbaceous creeping perennial. Photoperiod prior to vernalization is thus an important factor in the flowering response of R. acetosella. Changes in photoperiod provide reliable signals of seasonal change for plants (Davis, 2002; Searle and Coupland, 2004) and contribute to dormancy regulation and growth of many annual and perennial plant species (Arora et al., 2003; Heide, 2001; Leopold, 1951; Wareing, 1956). This signal was not provided to R. acetosella ramets maintained under 8 hour photoperiods prior to vernalization and may help
explain the low flowering percentage of these ramets, even when transferred to the 16 hour photoperiod following vernalization. Photoperiodism in plants is currently explained through external and internal coincidence models regulated by circadian clock-controlled gene expression and protein synthesis (Davis, 2002; Turck et al., 2008). Coincidence of light perception with peak expression of circadian clock-controlled genes triggers a physiological response in the plant (Davis, 2002). Photoperiods above a critical level, and subsequent decline below this level, must therefore play a physiological role in preparing *R. acetosella* ramets for the onset of vernalizing temperatures. A similar role has been suggested for temperature in regulating the vernalization response of *Euphorbia esula* (Foley et al., 2009). Identification of this critical photoperiod for growth prior to vernalization would be interesting as it would help determine if ramets emerging late in the season (Figs. 3.1, 3.2, and 4.2) undergo photoperiodic restrictions to vernalization and subsequent flowering. Finally, gradually decreasing photoperiod prior to vernalization increased the flowering response of *R. acetosella* (Table 5.17), though direct exposure to short days prior to vernalization may have been just as effective. Transfer of a variety of ornamental, food, and wild plants from long days to short days prior to vernalization increases the flowering response and also reduces the vernalization duration required to saturate the flowering response (Lopez and Runkle, 2006; Medd and Lovett, 1978; Rohwer and Heins, 2007; Yamasaki et al., 2000). Additional research should focus on this as it may provide a simple method for increasing the flowering response of *R. acetosella* ramets and reducing the duration of cold required in future experiments.

6.3.9. Role of Ramet Polycarpy in Reproduction of *Rumex acetosella*

Finally, ramets of herbaceous clonal perennials can exhibit annual, biennial and perennial growth habits (Tamm et al., 2002) that can affect ramet flowering frequency. Survival of
overwintering ramets was observed at all field sites (Figs. 3.5 and 3.6) and indicates a perennial habit of overwintering *R. acetosella* ramets that may contribute to ramet polycarpism. This is the first report of this potential phenomenon in ramets of this species. The maximum ramet life-span of the 32 herbaceous perennials classified as having perennial ramets by Tamm et al. (2002) ranged from 3-17 years. *R. acetosella* was not among the species studied and estimates of ramet life-span for this species are lacking. The life-cycle model developed in this thesis, however, allows for estimation of potential ramet life-span through prediction of the turnover rate of a given overwintering population. It is estimated that a net ramet population at the end of a given non-bearing year will decline to approximately 1% of that population within 3 years. The life-span of a ramet incorporated into the overwintering ramet population therefore likely ranges from 2-4 years in lowbush blueberry fields in Nova Scotia. Many overwintering ramets are therefore exposed to vernalizing conditions more than once in their life-span and may thus exhibit a polycarpic growth habit. Survival of flowering ramets was observed under both field (Figs. 3.5 and 3.6) and controlled conditions (Tables 5.6, 5.9, and 5.18) and re-vernality and subsequent flowering of these ramets could be explored through detailed tracking under field conditions or additional controlled experiments. Ramet polycarpy is reported for some herbaceous creeping perennials (Araki and Ohara, 2008; Eriksson, 1988; Pitelka et al., 1985), but has not been reported to occur in herbaceous perennials with a documented vernalization requirement for flowering.

6.4. General Conclusions

This thesis has resulted in the development of growing degree-day models for lowbush blueberry emergence and development and this provides a reliable basis for comparison of pest
development to that of the lowbush blueberry. Additional calibration and validation data sets could be collected to improve the precision of model parameters and growing degree-day thresholds, but the models developed in this thesis are currently adequate to aid management decisions in North-Central Nova Scotia. These are the first such models proposed for lowbush blueberry and this thesis now provides a framework for expansion of this approach to lowbush blueberry management in other regions.

The results of this thesis demonstrate for the first time that a demographic approach is effective for understanding the key processes and structure of perennial weed populations in lowbush blueberry fields. This approach can now be readily applied to other perennial weeds in this production system and should serve as a useful guide for the development of similar approaches in other perennial crops. Results of this thesis identified the role of overwintering and newly emerged ramets in the reproductive biology of *R. acetosella* in lowbush blueberry fields, and also identified the important role that seedlings likely play in the maintenance of genetic diversity in these populations. The proposed life-cycle model will serve as a useful tool for predicting and evaluating the impacts of management practices on ramet and genet dynamics and serve as a guide for the development of new management strategies. In addition, the growing degree-day models for predicting *R. acetosella* ramet emergence and flowering can be used to relate these processes to lowbush blueberry emergence and development. These models also serve as a useful tool for tracking ramets in future field studies on the ramet dynamics and biology of *R. acetosella*. 
The experiments on the flowering biology of *R. acetosella* have identified, for the first time, the important role of vernalization in the regulation of ramet development in this species. A good understanding of the effective temperature and duration of cold exposure required, the effective photoperiod for flower induction following vernalization, the role of pre-vernalization stimuli in regulating flowering, and the impacts of pre and post-vernalization ramet removal on flowering under both field and controlled conditions has been established. This is the most extensive work conducted on the flowering biology of this species and creates an opportunity for a variety of additional research regarding the role of vernalization in the regulation of flowering in herbaceous creeping perennials. The general guidelines for propagating and establishing *R. acetosella* under controlled conditions have been established and should be of great assistance to new students attempting additional studies with this species. The basic temperature and photoperiodic requirements for maintenance of vegetative ramets is provided, and conditions for successful induction of flowering have been confirmed. A logical progression of additional research is provided above and should provide fascinating insight into the regulation of ramet and genet development in herbaceous creeping perennials.
Chapter 7 : Literature Cited


Cook, R.E. 1983. Clonal plant populations. Amer. Sci. 71:244-253


Appendix A: Evaluation of MAT28 for crop tolerance and weed control in wild blueberry

A.1. Introduction

MAT28 is a new herbicide developed by DuPont for control of broadleaf weeds in a variety of cropping systems. Preliminary work with this product in wild blueberry has indicated potential efficacy on problematic weeds and good crop tolerance. However, official dose-response and large-scale screening trials have not been conducted to date.

The objectives of this research were to 1) conduct dose response trials for pre-emergence applications of MAT28 in wild blueberry, 2) evaluate pre-emergence applications of MAT28 for goldenrod control in wild blueberry, and 3) evaluate the effectiveness of MAT28 spot-spray applications on perennial weeds in wild blueberry

A.2. Materials and Methods

Blueberry Dose Response. A trial to evaluate the dose response of wild blueberry to pre-emergence applications of MAT28 was established at Debert and Londonderry, Nova Scotia, in May 2010. The experiment was a Randomized Complete Block Design with four replications. Plot size was 2 X 6m. Pre-emergence applications were made on May 5, 2010. Application rates were 0.5X, 1.0X, 2.0X, and 4.0X, where X is 100 g a.i. ha$^{-1}$. Herbicide applications were made with a 2 meter CO$_2$ pressurized plot sprayer equipped with four Teejet XR11002 nozzles. Weather conditions at the time of herbicide applications at each site are given in Table A.1.

Dose response was determined using crop biomass. Blueberry shoots in two 25 X 25cm quadrats were collected in each plot. Shoots were clipped at ground level, bagged in the field, and brought back to the lab and dried in an oven for 48 hours at 50°C. Mean biomass in each treatment was calculated from the two samples. Dose response curves were developed using the percent biomass reduction in each MAT28 treatment when compared to the control treatment. Additional data collection included herbicide damage ratings at 35, 56, and 77 DAS, blueberry shoot counts at 21, 35, and 56 DAS, blueberry shoot heights at 42 DAS, and blueberry flower bud number and shoot height of 20 stems per plot in late Autumn.
Table A.1. Application date, air temperature, wind speed, and relative humidity at time of MAT28 applications at Debert and Londonderry, Nova Scotia, in 2010.

<table>
<thead>
<tr>
<th>Site</th>
<th>Application Date</th>
<th>Temperature</th>
<th>Wind Speed</th>
<th>Relative Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debert</td>
<td>5 May 2010</td>
<td>16.0</td>
<td>2</td>
<td>49</td>
</tr>
<tr>
<td>Londonderry</td>
<td>5 May 2010</td>
<td>17.0</td>
<td>2</td>
<td>55</td>
</tr>
</tbody>
</table>

Goldenrod Screening Trial. A trial to evaluate MAT28 for preemergence control of goldenrods was established in Collingwood, Nova Scotia in May 2010. The experiment was a Randomized Complete Block Design with four replications. Plot size was 2 X 8m. Treatments evaluated are listed in Table A.2. MAT28 was applied alone and in tank mixes with other currently registered products. Post-emergent mesotrione applications were included as the industry standard. Herbicide applications were made with a 2 meter CO$_2$ pressurized plot sprayer equipped with four Teejet XR11002 nozzles. Weather conditions at time of herbicide applications are given in Table A.3.

Table A.2. Herbicide treatments for goldenrod screening trial at Collingwood, Nova Scotia, in 2010.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Active Ingredient (a.i.)</th>
<th>Application Rate g a.i. ha$^{-1}$</th>
<th>Application Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT28</td>
<td>Cycloaminopyralid</td>
<td>100</td>
<td>PRE$^z$</td>
</tr>
<tr>
<td>Velpar</td>
<td>Hexazinone</td>
<td>1960</td>
<td>PRE</td>
</tr>
<tr>
<td>Karmex</td>
<td>Diuron</td>
<td>1800</td>
<td>PRE</td>
</tr>
<tr>
<td>MAT28 + Velpar</td>
<td>See above</td>
<td>100 + 1960</td>
<td>PRE</td>
</tr>
<tr>
<td>MAT28 + Karmex</td>
<td>See above</td>
<td>100 + 1800</td>
<td>PRE</td>
</tr>
<tr>
<td>Callisto</td>
<td>Mesotrione</td>
<td>100</td>
<td>POST</td>
</tr>
<tr>
<td>Velpar + Callisto</td>
<td>See above</td>
<td>1960 + 100</td>
<td>PRE + POST</td>
</tr>
</tbody>
</table>

$^z$PRE, pre-emergence to goldenrods; POST, post-emergence to goldenrods
Table A.3. Application date, air temperature, wind speed, and relative humidity at time of PRE and POST herbicide applications at Collingwood, Nova Scotia, in 2010.

<table>
<thead>
<tr>
<th>PRE / POST</th>
<th>Application Date</th>
<th>Temperature °C</th>
<th>Wind Speed m s⁻¹</th>
<th>Relative Humidity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>5 May 2010</td>
<td>10.6</td>
<td>1.4</td>
<td>61</td>
</tr>
<tr>
<td>POST</td>
<td>9 June 2010</td>
<td>16.7</td>
<td>1.2</td>
<td>56.9</td>
</tr>
</tbody>
</table>

Data collection included herbicide damage ratings, goldenrod shoot counts, goldenrod shoot heights, and goldenrod biomass in each treatment. Damage ratings could not be normalized for the analysis of variance and are not presented. Goldenrod shoot counts were conducted at 14, 28, and 56 DAS for all PRE treatments. A final shoot count was also conducted on August 16 in all treatments. Only data from the final shoot count is presented due to the ability to compare data from all treatments. Goldenrod shoot heights were collected in all PRE treatments at 42 and 105 DAS, and goldenrod biomass was collected in all treatments on August 16. Goldenrod shoot counts were conducted in 2 1m X 1m quadrats per plot. Goldenrod shoot heights were measured on 20 shoots per plot. Goldenrod biomass was determined on 10 shoots per plot. Shoots were randomly selected, clipped at ground level, bagged in the field, brought back to the lab and dried in an oven for 48 hours at 50°C.

**Spot Spray Trials.** MAT28 was evaluated as a spot spray treatment on several perennial weeds in wild blueberry fields. Sites were established in Oxford, Collingwood, North River, and Antigonish, Nova Scotia, in mid to late summer 2010. MAT28 was applied at a rate of 1 g a.i. L⁻¹ water using a CO₂ pressurized sprayer equipped with a single Teejet 8002VS nozzle. A surfactant was not used. Weeds were sprayed until run-off of the herbicide solution from the leaves, and complete coverage of the target weed species was attempted in all applications. Ten individual plants of each species were treated, unless otherwise noted.
A.2.1. Data Analysis

Blueberry Biomass. Blueberry stem height, flower bud number, stem biomass, and stem density were analyzed using PROC MIXED in SAS. Means were determined using LSMEANS, and Tukey’s mean separation was used to identify significant differences between means. Data were transformed as required to ensure validity of the analysis of variance. Percent blueberry stem biomass reduction was plotted against MAT28 application rate, and a 3-parameter logistic model was fit to the data using the SigmaPlot™ graphing program. This was done separately for each site. Predicted values for percent biomass reduction were obtained from each model and were used to determine the MAT28 application rate which caused 50% reduction in crop biomass.

Goldenrod Screening Trial. Goldenrod biomass, shoot density, and shoot height data were analyzed using PROC MIXED. Means were determined using LSMEANS, and Tukey’s mean separation was used to identify significant differences between means.

MAT28 Spot Spray Trial. Damage ratings could not be normalized for means comparison in all species tested. Therefore, mean damage ratings, and standard error of the mean, were determined using PROC MEANS in SAS.

A.3. Results

Blueberry Dose Response Trial. The preliminary logistic model fitted to blueberry stem biomass data indicated a 50% reduction in biomass at a MAT28 application rate of 91 g a.i. ha\(^{-1}\) at each site (Fig. A.1). Reduction in biomass was significant at application rates of 100, 200, and 400 g a.i. ha\(^{-1}\), but decreased stem density was only significant at 200 and 400 g a.i. ha\(^{-1}\) (Table A.4). Reductions in final stem heights and flower bud numbers on surviving stems were only significant at the higher rates at Debert, but the 100 g a.i. ha\(^{-1}\) rate caused significant reduction in these parameters at Londonderry (Table A.5).
Fig. A.1. Percent blueberry stem biomass reduction in preemergence MAT28 treatments in sprout year wild blueberry fields at A) Debert and B) Londonderry, Nova Scotia. Closed circles are the mean percent biomass reduction in each treatment compared to the control treatment. Solid lines are the fitted 3-parameter logistic regression equation.
Table A.4. Blueberry stem biomass and density in MAT28 dose response trial at Debert and Londonderry, Nova Scotia, in 2010. Blueberry stem density data were log transformed for the analysis of variance. Transformed data are presented for means comparisons, and back-transformed data are given in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Debert</th>
<th></th>
<th>Londonderry</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blueberry Stem Biomass</td>
<td>Blueberry Stem Density</td>
<td>Blueberry Stem Biomass</td>
<td>Blueberry Stem Density</td>
</tr>
<tr>
<td>Control</td>
<td>6.9 ± 0.2 a (1019.4)</td>
<td>6.9 ± 0.2 a (969.4)</td>
<td>451.6 ± 35 a</td>
<td>6.9 ± 0.2 a</td>
</tr>
<tr>
<td>Velpar</td>
<td>6.9 ± 0.2 a (1047.9)</td>
<td>6.7 ± 0.2 a (804.2)</td>
<td>385.6 ± 35 ab</td>
<td>6.7 ± 0.2 a</td>
</tr>
<tr>
<td>MAT 0.5X</td>
<td>6.7 ± 0.2 ab (836.8)</td>
<td>6.8 ± 0.2 a (963.9)</td>
<td>302.0 ± 35 ab</td>
<td>6.8 ± 0.2 a</td>
</tr>
<tr>
<td>MAT 1.0X</td>
<td>6.4 ± 0.2 ab (700.7)</td>
<td>6.6 ± 0.2 a (781.9)</td>
<td>237.6 ± 35 b</td>
<td>6.6 ± 0.2 a</td>
</tr>
<tr>
<td>MAT 2.0X</td>
<td>6.2 ± 0.2 b (551.4)</td>
<td>6.2 ± 0.2 a (558.3)</td>
<td>45.0 ± 35 c</td>
<td>6.2 ± 0.2 a</td>
</tr>
<tr>
<td>MAT 4.0X</td>
<td>4.9 ± 0.2 c (151.4)</td>
<td>5.1 ± 0.2 b (184.7)</td>
<td>20.4 ± 35 c</td>
<td>5.1 ± 0.2 b</td>
</tr>
</tbody>
</table>

*Means within columns followed by the same letter do not differ significantly according to a Tukey’s mean separation test at the 0.05 level of significance.*
Table A.5. Blueberry stem heights and flower bud counts at Debert and Londonderry, Nova Scotia, in 2010.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stem Height</th>
<th>Flower Buds</th>
<th>Stem Height</th>
<th>Flower Buds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.8 ± 1 a²</td>
<td>4.3 ± 0.5 a</td>
<td>17.5 ± 0.7 a</td>
<td>4.7 ± 0.6 ab</td>
</tr>
<tr>
<td>Velpar</td>
<td>18.5 ± 1 a</td>
<td>4.6 ± 0.5 a</td>
<td>16.9 ± 0.7 a</td>
<td>6.2 ± 0.6 a</td>
</tr>
<tr>
<td>MAT 0.5X</td>
<td>17.0 ± 1 ab</td>
<td>4.0 ± 0.5 a</td>
<td>16.3 ± 0.7 ab</td>
<td>4.2 ± 0.6 abc</td>
</tr>
<tr>
<td>MAT 1.0X</td>
<td>17.1 ± 1ab</td>
<td>4.7 ± 0.5 a</td>
<td>13.4 ± 0.7 b</td>
<td>3.6 ± 0.6 bc</td>
</tr>
<tr>
<td>MAT 2.0X</td>
<td>12.7 ± 1 b</td>
<td>2.9 ± 0.5 ab</td>
<td>9.6 ± 0.7 c</td>
<td>2.4 ± 0.6 bc</td>
</tr>
<tr>
<td>MAT 4.0X</td>
<td>7.5 ± 1 c</td>
<td>1.7 ± 0.5 b</td>
<td>7.5 ± 0.7 c</td>
<td>2.2 ± 0.6 c</td>
</tr>
</tbody>
</table>

²Means within columns followed by the same letter do not differ significantly according to a Tukey’s mean separation test at the 0.05 level of significance.

Goldenrod Screening Trial. Goldenrod density in MAT28 treatments was reduced by about 62% compared to the control by late August (105 DAS), but surviving shoots did not have reduced biomass (Table A.6). Lack of biomass reduction of individual shoots seems to be due to recovery of surviving shoots from initial injury. Goldenrod shoot height was reduced by about 82% in MAT28 treatments in early summer, but heights of surviving shoots were similar to the control by 105 DAS (Table A.7). Shoot density was lowest in the mesotrione treatments, but surviving shoots recovered and biomass was similar to other treatments (Table A.6).

MAT28 Spot Spray Trial. Spot spray applications of MAT28 provided effective control of several perennial weed species in wild blueberry. Based on 56 DAS damage ratings, species controlled >80% included aralia (rosettes), birch, blackberry, spreading dogbane, honeysuckle, black knapweed, pincherry, evening primrose, raspberry, wild rose, and willow. Species controlled 50 – 80% included aralia (flowering), calico aster, maple, and St. John’s Wort. Species controlled < 50% were black bulrush, bunchberry, and pearly everlasting (Table A.8).
Table A.6. Goldenrod biomass and shoot density at 105 DAS in screening trial at Collingwood, Nova Scotia, in 2010.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Goldenrod Biomass</th>
<th>Goldenrod Shoot Density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g stem⁻¹</td>
<td>shoots m²</td>
</tr>
<tr>
<td>Control</td>
<td>1.5 ± 0.38 a</td>
<td>179.0 ± 15 a</td>
</tr>
<tr>
<td>MAT28</td>
<td>1.6 ± 0.38 a</td>
<td>62.3 ± 15 cd</td>
</tr>
<tr>
<td>Velpar</td>
<td>1.7 ± 0.38 a</td>
<td>155.3 ± 15 ab</td>
</tr>
<tr>
<td>Karmex</td>
<td>1.6 ± 0.38 a</td>
<td>210.3 ± 15 a</td>
</tr>
<tr>
<td>MAT28 + Velpar</td>
<td>1.8 ± 0.38 a</td>
<td>55.0 ± 15 a</td>
</tr>
<tr>
<td>MAT28 + Karmex</td>
<td>1.3 ± 0.38 a</td>
<td>84.3 ± 15 bc</td>
</tr>
<tr>
<td>Callisto</td>
<td>0.6 ± 0.38 a</td>
<td>7.0 ± 15 d</td>
</tr>
<tr>
<td>Velpar + Callisto</td>
<td>0.6 ± 0.38 a</td>
<td>6.4 ± 15 d</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter do not differ significantly according to a Tukey’s mean separation test at the 0.05 level of significance.

Table A.7. Goldenrod heights in PRE treatments at 42 and 105 DAS in MAT28 screening trial at Collingwood, Nova Scotia, in 2010. Data for 105 DAS were log transformed for the analysis of variance. Transformed data are presented for means comparisons, and back-transformed data are given in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>42 DAS</th>
<th>105 DAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td>cm</td>
</tr>
<tr>
<td>Control</td>
<td>22 ± 1.6 a</td>
<td>3.8 ± 0.1 ab (46)</td>
</tr>
<tr>
<td>MAT28</td>
<td>4 ± 1.6 b</td>
<td>3.5 ± 0.1 bc (32)</td>
</tr>
<tr>
<td>Velpar</td>
<td>21 ± 1.6 a</td>
<td>3.8 ± 0.1 a (47)</td>
</tr>
<tr>
<td>Karmex</td>
<td>21 ± 1.6 a</td>
<td>3.8 ± 0.1 ab (45)</td>
</tr>
<tr>
<td>MAT28 + Velpar</td>
<td>4 ± 1.6 b</td>
<td>3.5 ± 0.1 bc (33)</td>
</tr>
<tr>
<td>MAT28 + Karmex</td>
<td>4 ± 1.6 b</td>
<td>3.4 ± 0.1 c (30)</td>
</tr>
</tbody>
</table>

Means within columns followed by the same letter do not differ significantly according to a Tukey’s mean separation test at the 0.05 level of significance.
Table A.8. Weed species, height, growth stage, and herbicide damage ratings in MAT28 spot spray trial in 2010.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean Height</th>
<th>Growth Stage</th>
<th>Damage Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>---cm---</td>
<td></td>
<td>14 DAS</td>
</tr>
<tr>
<td>Aralia(^y)</td>
<td>77 ± 6.3</td>
<td>F(^z)</td>
<td>42 ± 3.7</td>
</tr>
<tr>
<td>Aralia</td>
<td>20 ± 3.8</td>
<td>R</td>
<td>96 ± 2.4</td>
</tr>
<tr>
<td>Birch</td>
<td>59 ± 5.6</td>
<td>V</td>
<td>27 ± 3.2</td>
</tr>
<tr>
<td>Blackberry</td>
<td>33 ± 2.6</td>
<td>V,FR</td>
<td>38 ± 5.3</td>
</tr>
<tr>
<td>Black Bulrush</td>
<td>98 ± 7.9</td>
<td>F</td>
<td>2.5 ± 0.8</td>
</tr>
<tr>
<td>Bunchberry</td>
<td>11 ± 0.8</td>
<td>V,F,FR</td>
<td>21 ± 2.3</td>
</tr>
<tr>
<td>Calico Aster</td>
<td>65 ± 3.0</td>
<td>V</td>
<td>50 ± 3.9</td>
</tr>
<tr>
<td>Spreading Dogbane</td>
<td>63 ± 2.6</td>
<td>V</td>
<td>90 ± 0.0</td>
</tr>
<tr>
<td>Honeysuckle</td>
<td>41± 3.6</td>
<td>V,F</td>
<td>24 ± 3.3</td>
</tr>
<tr>
<td>Black Knapweed</td>
<td>68 ± 2.9</td>
<td>F</td>
<td>36 ± 2.6</td>
</tr>
<tr>
<td>Maple</td>
<td>73 ± 5.5</td>
<td>V</td>
<td>13 ± 2.6</td>
</tr>
<tr>
<td>Pearly Everlasting</td>
<td>43 ± 4.2</td>
<td>F</td>
<td>8 ± 0.8</td>
</tr>
<tr>
<td>Pincherry</td>
<td>57 ± 3.5</td>
<td>V</td>
<td>55 ± 4.8</td>
</tr>
<tr>
<td>Evening Primrose</td>
<td>108 ± 5.8</td>
<td>B, F</td>
<td>60 ± 2.6</td>
</tr>
<tr>
<td>Raspberry</td>
<td>43 ± 4.6</td>
<td>V</td>
<td>56 ± 6.0</td>
</tr>
<tr>
<td>St. John’s Wort</td>
<td>70 ± 2.1</td>
<td>F</td>
<td>4 ± 0.7</td>
</tr>
<tr>
<td>Wild Rose</td>
<td>40 ± 2.1</td>
<td>V,F,FR</td>
<td>70 ± 0.0</td>
</tr>
<tr>
<td>Willow</td>
<td>74 ± 4.4</td>
<td>V</td>
<td>67 ± 3.2</td>
</tr>
</tbody>
</table>

\(^z\) F, flowering; FR, Fruiting; R, rosette; V, vegetative; B, budding

A.4. Summary

Preemergence MAT28 applications significantly reduced blueberry stem biomass m$^{-2}$. A 50% reduction in biomass was found to occur at 91 g a.i. ha$^{-1}$. Reductions in stem height and flower buds on surviving stems were variable at rates up to 100 a.i. ha$^{-1}$, but consistent reductions occurred at higher rates tested. Preemergence applications of MAT28 at 100 g a.i. ha$^{-1}$ significantly reduced goldenrod density, but biomass of surviving shoots was similar to the control. Spot spray applications of MAT28 at a rate of 1g a.i. L water$^{-1}$ controlled top-growth of several perennial weeds in wild blueberry fields.