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Seasonal Development of the Biological Control Agent of Dalmatian Toadflax, Mecinus janthiniformis (Curculionidae: Coleoptera), in Utah: Phenology, Overwintering Success, and Mortality

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SEASONAL DEVELOPMENT OF THE BIOLOGICAL CONTROL AGENT OF DALMATIAN TOADFLAX, *MECINUS JANTHINIFORMIS* (CURCULIONIDAE: COLEOPTERA), IN UTAH: PHENOLOGY, OVERWINTERING SUCCESS, AND MORTALITY

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE in

Ecology

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ABSTRACT

Seasonal Development of the Biological Control Agent of Dalmatian Toadflax, 
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Overwintering Success, and Mortality

by

Samantha A. Willden, Master of Science 
Utah State University, 2017

Major Professor: Dr. Edward W. Evans  
Department: Biology

Invasive weeds are threatening ecosystem function and productivity all over the world by outcompeting desirable vegetation and reducing species diversity. One option for long-term control of such weeds is biological control using natural insect enemies. Such a program has been developed for management of Dalmatian toadflax (*Linaria dalmatica* (L.) Miller (Plantaginaceae)) in North America using a stem-mining weevil, *Mecinus janthiniformis* Toševski and Caldara (Coleoptera: Curculionidae). Although widely effective in northern regions, such as in British Columbia and in the American northwest, this insect has been slow to suppress Dalmatian toadflax in southern most regions of their current range, including areas in Utah and Colorado, and little is known of the limiting factors leading to slow weed suppression in these areas.

Using field assessments of insect and plant activity over two growing seasons at several sites, this study aimed to provide degree-day and calendar-date descriptions of
insect phenology. In addition, dead, overwintered Dalmatian toadflax stems were dissected to determine overwintering mortality of weevil adults before spring emergence, and living stems were dissected to determine development stages and mortality of weevils during summer development.

Degree-day and calendar-date based models independently resulted in consistent trends in weevil phenology between sites and between years in this study, although the degree-day model is likely to be most useful for purposes of predicting weevil life cycle timing. Interestingly, the sexes differed in their phenology in that males consistently emerged from overwintering sites and were found on Dalmatian toadflax stems considerably earlier than females in the spring. Females as well as males tended to peak in abundance on stems in late-May when Dalmatian toadflax stems reached full maturity.

Overall mortality of *M. janthiniformis* during a lifecycle was low for all samples; approximately 83% of adults successfully emerged from overwintered stems in the following spring, and greater than 65% of larvae survived to adulthood before overwintering. This resulted in >50% of weevils surviving larval development, overwintering, and spring emergence as adults. The majority of *M. janthiniformis* deaths (51%) resulted from parasitism by chalcidoid wasps during summer development to adulthood. These parasitoid wasps, and also *M. janthiniformis* adults, likely created the peculiar exit holes that were observed in live Dalmatian toadflax stems during the summer.

Although *M. janthiniformis* populations were slow to provide effective control of Dalmatian toadflax at sites in Utah, this study indicates that the phenology and
survivorship of *M. janthiniformis* individuals in Utah are well suited for successful biocontrol. If given enough time to build populations, *M. janthiniformis* appears to be capable of providing effective Dalmatian toadflax control in southern regions, but other limiting factors, such as precipitation and host plant quality, should be considered in future studies to explain slow weed suppression. Phenology models and estimates of mortality of *M. janthiniformis* generated by this study at sites in Utah may be helpful in implementing future biocontrol programs of Dalmatian toadflax.

(171 pages)
PUBLIC ABSTRACT

Seasonal Development of the Biological Control Agent of Dalmatian Toadflax, *Mecinus janthiniformis* (Curculionidae: Coleoptera), in Utah: Phenology, Overwintering Success, and Mortality

Samantha A. Willden

By outcompeting desirable vegetation, invasive weeds can dominate field crops and rangelands, drastically reducing yield and land value. One option in controlling the impact and spread of such weeds is reuniting them with their natural insect herbivores, a process called biological control. When successful, biocontrol can be the cheapest way to provide long-term control of invasive weeds, but continual monitoring of insect and weed activity is required to ensure success.

Dalmatian toadflax is an invasive weed that occurs widely throughout the northwestern U.S., and that is spreading south each year to warmer and drier regions, including sites in Utah. Although successful in the northwest, biocontrol of Dalmatian toadflax using a stem-mining weevil, *Mecinus janthiniformis* Toševski and Caldara (Coleoptera: Curculionidae), has been slow to occur at sites in Utah and elsewhere in the weevil’s current southern range. By making field assessments of insect activity at sites in Utah, this study aimed to evaluate the limitations of weed control in these southern regions including inadequate timing of biological events (phenology) and the mortality of adult weevils during the winter and of individuals during summer development to adulthood.
This study found that weevils at sites in Utah were synchronized well with the biology of Dalmatian toadflax, but the sexes differed in their phenology in that males emerged from overwintering sites considerably earlier than females (a phenomenon called protandry). Overall survival of weevil adults during winter, and larvae during summer development to adulthood was high, (83% and 65%, respectively). The majority of *M. janthiniformis* deaths (51%) in live stems during the summer were the result of attack by parasitoid wasps. These wasps, and adult weevils, were found in association with exit holes observed in live Dalmatian toadflax stems during the summer. Overall survival of weevils from larval development in the summer, to adult emergence from overwintered stems in the following spring, was >50%.

Although suppression of Dalmatian toadflax was slow to occur at Utah sites, this study indicates that the phenology and low mortality of *M. janthiniformis* in Utah should contribute to effective biocontrol. Although other factors that may limit weed control were not considered in this study, *M. janthiniformis* appears to be capable of surviving and controlling Dalmatian toadflax in southern regions of North America. Phenology models and estimates of mortality of *M. janthiniformis* generated in this study can contribute to the implementation of future biocontrol control programs for Dalmatian toadflax.
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Samantha A. Willden
# CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>PUBLIC ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>9</td>
</tr>
<tr>
<td>II. PHENOLOGY OF THE BIOLOGICAL CONTROL AGENT OF Dalmatian Toadflax, <em>Mecinus Janthiniformis</em> (Curculionidae: Coleoptera), in Utah</td>
<td>14</td>
</tr>
<tr>
<td>Abstract</td>
<td>14</td>
</tr>
<tr>
<td>Introduction</td>
<td>15</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>21</td>
</tr>
<tr>
<td>Results</td>
<td>30</td>
</tr>
<tr>
<td>Discussion and Conclusions</td>
<td>38</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>49</td>
</tr>
<tr>
<td>Tables and Figures</td>
<td>56</td>
</tr>
<tr>
<td>III. OVERWINTERING SUCCESS IN UTAH OF <em>Mecinus Janthiniformis</em> Adults, a Biological Control Agent of Dalmatian Toadflax</td>
<td>74</td>
</tr>
<tr>
<td>Abstract</td>
<td>74</td>
</tr>
<tr>
<td>Introduction</td>
<td>75</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>77</td>
</tr>
<tr>
<td>Results</td>
<td>79</td>
</tr>
<tr>
<td>Discussion and Conclusions</td>
<td>83</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>88</td>
</tr>
<tr>
<td>Tables and Figures</td>
<td>92</td>
</tr>
<tr>
<td>IV. DEVELOPMENT OF THE TOADFALX STEM-BORING WEEVIL, <em>Mecinus Janthiniformis</em>, from Larva to Adulthood: Phenoology Models, Mortality, and Implications on Weed Control</td>
<td>107</td>
</tr>
<tr>
<td>Abstract</td>
<td>107</td>
</tr>
</tbody>
</table>
Introduction ........................................................................................................... 108
Methods and Materials ......................................................................................... 111
Results .................................................................................................................... 117
Discussion and Conclusions .................................................................................. 125
Literature Cited ...................................................................................................... 135
Tables and Figures .................................................................................................. 139

V. CONCLUSIONS .................................................................................................. 148

APPENDICES ........................................................................................................... 153
Appendix. Examples of exit holes found in live Dalmatian toadflax stems, and photos of parasitoids of M. janthiniformis ...................................................... 154
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Results of a two-way ANOVA on the effects of site and year on the number of <em>M. janthiniformis</em> adults observed on the host plant during peak abundance</td>
<td>56</td>
</tr>
<tr>
<td>2.2</td>
<td>Results of a $\chi^2$ test used to compare the sex ratio over the season at Kennecott and Lake Point combined in 2014 and at Lake Point in 2015. Individual tests were conducted for females versus males as grouped into six time periods in each year</td>
<td>57</td>
</tr>
<tr>
<td>2.3</td>
<td>Results of an ANCOVA of reproduction (flowering vs. non-flowering stems) and height (cm) as a covariate on the number of male and female individuals found on whole stems at Pine Canyon on May 19, 2016</td>
<td>58</td>
</tr>
<tr>
<td>2.4</td>
<td>Results of two ANCOVAs of reproduction (flowering vs. non-flowering stems) and height (cm) as a covariate on the number of total individuals (males and females) or females only found on the top 10 cm of stems observed at Pine Canyon on May 19, 2016</td>
<td>59</td>
</tr>
<tr>
<td>2.5</td>
<td>Results of an ANCOVA of reproduction (flowering vs. non-flowering stems) and height (cm) as a covariate on the number of mating pairs observed on whole stems and on the top 10 cm of stems measured on 19 May 2016 at Pine Canyon</td>
<td>60</td>
</tr>
<tr>
<td>3.1</td>
<td>Sites and dates of overwintered stem collections made in 2014 and 2015</td>
<td>92</td>
</tr>
<tr>
<td>3.2</td>
<td>Summary of ANOVA results comparing abundance of weevils in stems collected at early, mid and late intervals during 2014 or 2015 at several sites</td>
<td>92</td>
</tr>
<tr>
<td>3.3</td>
<td>Results of four paired t-tests comparing the numbers of males and females (alive or dead) present in overwintered stems collected at several sites in March of 2014 and 2015</td>
<td>93</td>
</tr>
<tr>
<td>3.4</td>
<td>Results of a two-way ANOVA of the effect of sample (site-year) and date of collection (March or May) on the percentage of individuals in a stem that were female</td>
<td>93</td>
</tr>
<tr>
<td>3.5</td>
<td>Results of four paired t-tests comparing the numbers of dead male and female adults present in overwintered stems collected in June/July of 2014 and 2015 at several sites</td>
<td>94</td>
</tr>
</tbody>
</table>
3.6 Results from ANCOVA of the effects of sample (site-year) and density on the percentage of adults that were dead by late season stems. A sqrt arcsine transformation was applied to the percentage ........................................94

3.7 Results from ANCOVA for each sex of the effect of sample (site-year) and stem density on the average body size of weevils that were alive or dead occurring within March stems.........................................................95

3.8 Results from ANOVA of the effect of status (live or dead) and sample (site-year) on male or female adult body size. Live individuals from March stems are compared against individuals as combined from all early, mid, and late season samples of stems.........................................................96

3.9 Results from ANOVA of the effect of date of collection (mid or late season stem samples) and sample (site-year) on male or female dead adult body size. ..................................................................................................................97

3.10 Results of two ANOVAs of the effect of emergence hole presence and sample (site-year) on dead adult body size. Dead adults from mid and late season samples are combined for each sample. .................................................98

4.1 Results of a one-way ANOVA analysis determining the effect of sampling date on stem density of developing weevils during the 2015 and 2016 seasons ...........................................................................................................139
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Average number of <em>M. janthiniformis</em> on the host plant at two sites over the 2014 and 2015 growing seasons from March 11 (Julian day of year 70) to July 9 (Julian day of year 190).</td>
<td>61</td>
</tr>
<tr>
<td>2.2</td>
<td>Degree-day accumulation (as determined using the single sine method with a critical base temperature of 8.9°C and a cutoff threshold of 28.7°C) in 2014 and 2015 as a function of Julian day of year. Day of year 200 corresponds to 8 July.</td>
<td>62</td>
</tr>
<tr>
<td>2.3</td>
<td>The effect of heat accumulation on the proportion of males in field collections made throughout the 2014 and 2015 seasons. $R^2 = 0.93$ and $p &lt; 0.001$ for 2014 and 2015.</td>
<td>63</td>
</tr>
<tr>
<td>2.4</td>
<td>Population dynamics of male and female <em>M. janthiniformis</em> adults as a function of degree-day accumulation for 2014 and 2015. Percentage of maximum represents the abundance of individuals on each sample date divided by the maximum abundance observed at that site and year.</td>
<td>64</td>
</tr>
<tr>
<td>2.5</td>
<td>Relative abundance of male and female <em>M. janthiniformis</em> adults by Julian day of year in 2014 (top) and 2015 (bottom) at Lake Point S (left) and Pine Canyon (right). Individuals per stem at 2014 sites were estimated using the regression line in Fig. 2.3.</td>
<td>65</td>
</tr>
<tr>
<td>2.6</td>
<td>A comparison of stem height (A), stem density (B), and flowering activity (C) of Dalmatian toadflax at two sites over the 2014 and 2015 growing seasons. Filled arrows represent peak female abundance in 2015 while unfilled arrows represent predicted peak female abundance in 2014.</td>
<td>66</td>
</tr>
<tr>
<td>2.7</td>
<td>A linear regression of the effect of stem height on the abundance of weevils on individual stems, with the sexes combined on May 21-22 of 2014 and 2015.</td>
<td>67</td>
</tr>
<tr>
<td>2.8</td>
<td>Linear regression of the number of <em>M. janthiniformis</em> observed on stems of varying height at two sites in June of 2015 (top) and at Pine Canyon in 2016 (bottom).</td>
<td>68</td>
</tr>
</tbody>
</table>
| 2.9 | Linear regressions of the effect of stem height on the number of male (right) and female (left) weevils present on each stem at Lake Point (circles) and Pine Canyon (triangles) in 2015. Data was collected on the
22 May 2015 for Lake Point and on 21 May 2015 for Pine Canyon........69

2.10 The effect of stem height on the numbers of males (right) or females (left) observed on stems on 8 June 2015 at Lake Point (top) and Pine Canyon (bottom)..............................................................70

2.11 A histogram showing the distribution of stems on 8 June 2015 at Lake Point (top N=33) and Pine Canyon (bottom N=40) having 0-16 females (left) or 0-16 males (right) ...............................................................71

2.12 Linear regressions of the effect of plant height on the total number of adults (A; males and females) and females only (B) on the top 10 cm of corresponding stems. Filled and unfilled triangles represent the flowering status of a particular stem as being flowering or not flowering (i.e. “non-flower”). Data was collected on May 19, 2016 at Pine Canyon ....72

2.13 The effect of stem height on the number of mating pairs present on the whole stem (A) on the on the top 10 cm of each stem (B) ..................73

3.1 Percentage of weevils that were identified as dead, emerged, or alive in stem samples collected on different dates at several sites in 2014 and 2015. N=30 stems for each sample..............................................................99

3.2 Average percentage (±SE) of live individuals that were females within overwintered stems collected in March (dark gray) and May (light gray) at several sites in 2014 and 2015. N refers to the number of live females recovered from 30 dissected stems for each sample........................................100

3.3 Comparison of the average number of dead male and female adults recovered from overwintered stems collected at three times of the spring and early summer during 2014 and 2015 at several sites. ..................101

3.4 Percentage of dead males or females recovered from late season stem collections that had chewed an exit hole before dying. N refers to the total number of dead males or females recovered with an exit hole. To obtain larger sample sizes, additional stems were collected at Lake Point in 2014 (resulting in 60 stems dissected) and at Pine Canyon in 2015 (resulting in 120 stems dissected).........................................................102

3.5 Regression of the number of weevils in a stem (“stem density”) and death rate of weevils (square root arcsine transformation of the percentage of dead individual) for stems collected in late season samples at two sites (Lake Point left circles and Pine Canyon right triangles) in 2014 (unfilled data points) and 2015 (filled data points) ........103
3.6 Histogram plotting the frequency of individuals in size categories, ranging from 3-5.5 mm, recovered from stems collected in March of 2014 and 2015 at two sites. Each individual measurement is rounded to the nearest quarter of a millimeter .......................................................... 104

3.7 Comparison of adult body size of those identified as dead (collections from early, mid, and late-season stems combined) and alive within stems collected in March, from several sites in 2014 and 2015. Asterisks represent p-values <.05 when comparing live and dead individuals in average body size ........................................................................... 105

3.8 Comparison of body size of dead adults in stems collected at mid or late-season in 2014 and 2015 at several sites .................................................. 106

4.1 Relative proportions of developing larvae, pupae and adults of those recovered alive in stems collected during the 2015 (A) and 2016 (B) seasons at Pine Canyon ................................................................. 140

4.2 The percentage of individuals found dead of the total number of individuals recovered in stem samples collected during the 2015 (A) and 2016 (B) seasons at Pine Canyon. Dead includes all dead larvae, pupae, adults and those parasitized .......................................................... 141

4.3 The average relative densities of living *M. janthiniformis* individuals within the larval, pupal, and adult life-stages per stem collected over the 2015 (top) and 2016 (bottom) seasons at Pine Canyon. Stems in 2015 were dissected under a microscope while those in 2016 were dissected by eye .................................................................................................................. 142

4.4 Average density of dead weevils (life stages combined) by degree-day accumulation in stems collected during the 2015 (solid line) and 2016 (dotted line) seasons ................................................................................. 143

4.5 Proportions of dead weevils that died as larvae, pupae, adults, or were presumed parasitized in stems collected during the 2015 season ............. 144

4.6 Average number of parasitized weevils by degree-day accumulation in stems collected during the 2015 season ......................................................... 145

4.7 Life-stage comparison of parasitoid wasps recovered in 2015 stems including those that died and those that exited stems ................................. 146

4.8 Average density of total exit holes observed on stems collected during 2015 at Pine Canyon by degree-day accumulation (A) and of that the average number of exit holes chewed by *Mecinus janthiniformis* and parasitoid wasps (B) ................................................................................. 147
A.1 Examples of exit holes chewed by adult parasitoid wasps (1) and by 
*M. janthiniformis* adults (2) ........................................154

A.2 Photos of *Mecinus janthiniformis* parasitoids including (1) a 
Pteromalidae endoparasitoid adult, (2) a Pteromalidae ectoparasitoid 
larva, and (3) an adult Eupelmidae wasp...............................155
CHAPTER 1

INTRODUCTION

Invasive and non-indigenous species are rapidly increasing in number worldwide, threatening the composition and functioning of natural ecosystems by outcompeting and ultimately replacing desirable species. Without proper control, invasive species cause severe ecological and economic damage; in the United States alone invasive species threaten 50% of imperiled species and cost 120 billion dollars per year in yield loss and environmental damage to agriculture (Leung et al. 2002, Mack et al. 2000, Pimentel et al. 2005, Wilcove et al. 1998). Among the most threatening invasive species, land plants are the dominant group, accounting for roughly 32% of the “world’s worst invasive alien species” listed by Luque et al. (2014). This is not surprising, as plants have astounding reproductive capacities, allelopathic competition, profound genetic diversity, and other characteristics leading to successful dispersal and survival in novel territories.

Dalmatian toadflax (*Linaria dalmatica* (L.) Miller (Plantaginaceae)), is an herbaceous invasive weed in North America that is especially threatening to rangeland plant communities. It was first introduced to North America by the late 1800s from Eurasia as a desirable ornamental plant often used in folk remedies (Alex 1962, Sing et al. 2016). Due to its charismatic resemblance to wild snapdragon, Dalmatian toadflax was originally planted in flowerbeds, but soon spread to unfavorable locations (Jeanneret and Schroeder 1992). Currently, Dalmatian toadflax has established well in every province in Canada and in the northwestern U.S., and land managers are on high alert in southern regions as it spreads to southwestern U.S. states including areas in Utah, Arizona,

Dalmatian toadflax is an especially pernicious weed due to its perennial lifecycle, bimodal reproduction (reproduction by seeds and vegetative root buds), prolific seed production, toxicity to livestock and extensive root systems (Alex 1962, Robocker 1974, Vujnovic and Wein 1997, Jeanneret and Schroeder 1992, Jamieson and Bowers 2010). In addition, Dalmatian toadflax can tolerate a wide range of temperatures and soil types, although preferred habitats tend to be in disturbed semi-arid areas with coarse textured soils up to 2800 m above sea level (Vujnovic and Wein 1997, Alex 1962). These characters ultimately lead to the successful establishment of Dalmatian toadflax in open, disturbed areas and the displacement of desirable vegetation (Alex 1962, Robocker 1974, Vujnovic and Wein 1997, Sing and Peterson 2011). If not properly controlled, Dalmatian toadflax can spread rapidly and reduce rangeland value (De Clerck-Floate and Harris 2002).

Recommendations for the control of Dalmatian toadflax on rangelands are limited due to the size of infestation and cost of treatment. Large infestations of Dalmatian toadflax are not often controlled using chemical or mechanical methods due to cost and difficulty in application (Sing et al. 2008, Park 2013). Herbicide treatment for Dalmatian toadflax is especially expensive in rangelands, where Dalmatian toadflax can cover
expanse areas of land. Tordon 22K (active ingredient picloram) is most commonly used to treat Dalmatian toadflax, and is often applied at $40/acre (recommended application of 2 quarts per acre) every 3 to 12 years to provide effective long-term weed control (Park 2013, South Dakota State University 2010). Other effective herbicides include active ingredients such as dicamba, chlorsulfuron, aminocyclopyrachlor, and imaxapic (USDA 2014). A study by Kyser and DiTomaso (2013) found aminocyclopyrachlor to be an effective alternative to picloram in California, providing control for 2 years. Mechanical control of Dalmatian toadflax is potentially less expensive, but likely less effective due to the terrain Dalmatian toadflax occupies, its deep tap root systems, and the potential to spread seeds. Burning is also not a recommended option, as burning has little impact on vegetative roots and could potentially leave burned landscapes vulnerable to new weed infestations (Anthony 2005, Sing et al. 2008).

In general, mechanical and especially chemical control is recommended for immediate treatment of small Dalmatian toadflax infestations to prevent further spread and colonization. However, these control tactics are usually infeasible in rangelands where Dalmatian toadflax is well established and covers vast areas of land. In these cases, chemical and mechanical advances have so far been largely unsuccessful in controlling and suppressing Dalmatian toadflax (Sing et al. 2016). In contrast, biological control practices using a stem-mining weevil, *Mecinus janthiniformis* Toševski and Caldara sp.n. (Coleoptera: Curculionidae), have been largely effective in providing large-scale and long-term suppression of Dalmatian toadflax in Canada and in the northwestern United States (De Clerck-Floate and Harris 2002, Carney 2003, Park 2013, Van Hezewijk et al. 2005).
2010, Schat et al. 2011, Sing et al. 2008, Peterson et al. 2005). However, biological control of Dalmatian toadflax populations at sites in the weevil’s most southern regions, including sites in Utah, have not been as successful (see below) (Jamieson et al. 2012, A. Mendenhall, USDA-APHIS, personal communication).

Many invasive and damaging pests are nonindigenous species that have traveled, largely alongside humans, to uncolonized areas where they have the potential to establish and spread precipitously. A major hypothesis used in explaining the success of non-native species in novel habitats is the “enemy release hypothesis,” which predicts that nonindigenous species may be highly competitive because they have been suddenly “released” from the predation and pressures of their natural enemies (Keane and Crawley 2002, Colautti et al. 2004). Without these enemies, invasive pests are free to establish, survive and spread in novel territories, often at the detriment of native species. The aim of classical biological control programs, centered on this hypothesis, is to reunite invasive pests with their natural co-evolved enemies from their native ranges, with the goal of “restoring the ecological balance” of pest populations that is experienced in their native ranges (Sing et al. 2016, DeBach 1964, Keane and Crawley 2002). These natural enemies or “biocontrol agents” can indeed be very effective in providing long-term and sustainable suppression of pest populations, and are often host specific to reduce negative impacts on neighboring species (Caltagirone 1981).

So far, nine species of biocontrol insects have been intentionally or unintentionally introduced to feed on noxious toadflaxes in North America (Sing et al. 2016, Winston et al. 2014). All are within the orders Coleoptera and Lepidoptera (beetles
and moths, respectively) and five were pre-screened for suitability and host specificity by CABI Bioscience before introduction to North America (Sing et al. 2016). Of these, the most successful biocontrol agent was *Mecinus janthiniformis*, a stem-mining weevil approved for release in British Columbia in 1991 as a biocontrol agent of Dalmatian toadflax (Jeanneret and Schroeder 1992, Harris et al. 2000, Nowierski 2004, Sing et al. 2016).

Recently distinguished from the yellow toadflax feeding weevil *Mecinus janthinus* Germar (Coleoptera: Curculionidae), *Mecinus janthiniformis* is native to former Yugoslavia and is host specific in feeding and developing exclusively on Dalmatian toadflax plants (Toševski et al. 2011). This species was first introduced along with *M. janthinus*, but was not distinguished as a separate species until 2011 (Toševski et al. 2011). Adults of *M. janthiniformis* are recognized by their by elongated, oval and black-metallic bodies approximately 4 mm in length and are often found feeding on foliage of Dalmatian toadflax throughout a growing season. Adults mate from May to early-June (Toševski et al. 2011), after which each female will lay approximately 1.15 eggs per day over the course of 2 months (De Clerck-Floate and Miller 2002). Eggs generally hatch within one week of oviposition inside stems and the larvae feed internally on stem tissue, resulting in the creation of hollow mines 1-3 cm long that also serve as dwellings for pupation and overwintering (Sing et al. 2016). After pupation, adults spend the winter inside Dalmatian toadflax stems until emergence in the following spring. Thus, this lifecycle takes one year to complete.
Internal mining by larvae and external feeding by adults of *M. janthiniformis* can have substantial impacts on Dalmatian toadflax growth and reproduction. During a growing season, this feeding damage can cause significant reductions in stem height, stem density and flower production, leading to plant suppression once impact or “outbreak” populations have been established (De Clerck-Floate and Harris 2002, Carney 2003, Park 2013, Van Hezewijk et al. 2010). Because weevils have the ability to spread naturally through Dalmatian toadflax stands (Van Hezewijk et al. 2010) and can survive in varied environments (Park 2013), biocontrol using *M. janthiniformis* is considered to be the most inexpensive and effective method for Dalmatian toadflax control on rangelands, amounting to less than $5/acre when considering the initial process of testing, rearing, and establishing insect population for release (Park 2013, Cate 1990, Harris et al. 2000, Nowierski 2004).

Although biocontrol programs can be effective, their implementation to combat invasive weeds is a long and laborious process, of which success is often unpredictable (Gurr and Wratten 2000, Hokkanen 1985, Williamson 1996, Julien 1997). In the case for Dalmatian toadflax, biological control using *M. janthiniformis* has been largely successful in Canada and in the northwestern United States; however, weevils have been slow to establish and provide effective weed control of Dalmatian toadflax in newly infested southern regions, including sites in Utah, that are characterized by hot/dry summers and low elevations (Jamieson et al. 2012, A. Mendenhall, USDA-APHIS, personal communication). There are likely many complex biotic and abiotic factors that influence a biocontrol agent’s ability to establish and thrive after introduction to a new
area. Among others, two “preconditions” required for biocontrol success include life cycle synchronization of biocontrol agents and hosts (Sing et al. 2016, Crawley 1989, Shepherd 1995, Harris and Zwölfer 1968) and favorable environmental conditions that facilitate survival and establishment of biocontrol agents. Climatic variables indeed vary between northern regions where *M. janthiniformis* has provided effective weed control and southern regions where *M. janthiniformis* has been slow to establish. Warmer and drier climates of southern regions may have negatively influenced the “preconditions” required for successful biocontrol in these areas, and particularly the phenology and survival of weevils.

Overall, little is known of these limiting factors and others experienced by weevils in southern regions that may have inhibited biocontrol success. To help unravel this mystery, this study aimed to directly investigate the phenology and mortality of weevils at southern sites in Utah where weevil establishment has been slow to occur. The results of this study may help explain slow weed suppression in the region, and may also provide helpful insights for future biocontrol programs.

Adult phenology is addressed in Chapter 2 using degree-day and calendar-dating models to describe adult presence and abundance on Dalmatian toadflax stems during a growing season. If southern regions have warmer spring conditions than northern regions where *M. janthiniformis* is well-established, one may expect shifts in *M. janthiniformis* phenology leading to earlier spring emergence and potential asynchrony with Dalmatian toadflax biology. Mortality of *M. janthiniformis* individuals is first addressed in Chapter 3 as overwintering and spring mortality of adults occurring before stem emergence, and
next in Chapter 4 as mortality of individuals during spring and summer development into adulthood. Estimates of overall mortality of *M. janthiniformis* during its lifecycle vary considerably in previously published studies (Jeanneret and Schroeder 1992, Toševski et al. 2011, Sing et al. 2016, De Clerck-Floate and Miller 2002, Schat 2008, Sing et al. 2008), so the estimates from the present study can further shed light on these previous observations. In addition to assessing mortality, Chapter 4 also includes phenology models, based on degree-days, of developing individuals from larvae to adulthood within Dalmatian toadflax stems. Several studies have previously investigated *M. janthiniformis* development to adulthood, but only in northern regions and only as based on calendar dates (Jeanneret and Schroeder 1992, Toševski et al. 2011, Sing et al. 2016, McClay and Hughes 2007).

My hypotheses at the beginning of these studies were: 1) the phenology of *M. janthiniformis* at the study sites in Utah would differ from findings in northern regions, namely in that adults would emerge earlier from overwintering sites, and with faster developmental rates, but there would still be adequate synchronization with Dalmatian toadflax biology, 2) degree-day models of insect phenology would demonstrate consistent results between sites and years in this study while results from calendar dating models would be more variable, and 3) mortality of *M. janthiniformis* individuals at the study site in Utah would differ from observations in northern regions in that during a growing season, developing weevils would have higher rates of mortality in Utah due to hot and dry summer conditions, while during overwintering, adults in Utah would have
lower rates of mortality compared to northern regions due to exposure to milder winter conditions.

Overall, I predicted that the phenology and mortality of *M. janthiniformis* individuals would be well-suited for effective biocontrol of Dalmatian toadflax in southern regions. Using data collected from field surveys and from stem dissections, I tested my hypotheses while generating useful models that may be helpful in predicting mortality or phenology of *M. janthiniformis* at sites in the future.

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CHAPTER 2

PHENOLOGY OF THE BIOLOGICAL CONTROL AGENT OF DALMATIAN TOADFLAX, *MECINUS JANTHINIFORMIS* (CURCULIONIDAE: COLEOPTERA), IN UTAH

Abstract

Noxious weeds threaten biodiversity and ecosystem function in range and wild lands by outcompeting and ultimately displacing desirable vegetation. One option for the control of such weeds is biological control via insect herbivory. Insects have been introduced as natural biocontrol agents of many pervasive weeds in North America but there is limited understanding of their phenology or timing of life stages. Presented is a study based on the phenology of *Mecinus janthiniformis* Toševski and Caldana attack on *Linaria dalmatica* (L.) Mill. in Utah. Stem census and sexing data were collected for two consecutive years on the host plant in Tooele, Utah to compare general population and sex specific seasonality using simple calendar dating and degree-day modeling. The results showed consistent phenological patterns of *M. janthiniformis* populations at individual sites between years while there were differences in phenology between the sexes in that males appeared earlier on the host plant than females, an example of insect protandry. Although males reached peak abundance slightly earlier than females in one year and considerably earlier in the second, overall patterns of phenology of each sex were fairly consistent when assessed using degree-days. Thus degree-day modeling, as opposed to calendar dating, proved to be the more reliable method for modeling.
phenology. An important application of this study was the development of degree-day predictions of weevil phenology that could be useful for guiding practitioners of biocontrol in determining when to visit *L. dalmatica* populations to assess agent establishment and to collect insects for future distribution.

**Introduction**

The impact of invasive species in agriculture and native communities is a primary concern in land management all over the world. Invasions by these species can lead to reduced functional and species diversity, and potentially undesirable novel ecosystems (Sala et al. 2000, Mack et al. 2000, Belnap et al. 2012). In agroecosystems, including rangelands, noxious weeds disrupt ecosystem services by outcompeting and ultimately displacing desirable vegetation, such as crops and forage plants, leading to severe economic loss and habitat degradation (Paini et al. 2016, DiTomaso 2000). Indeed, exotic plants are invading 700,000 hectares per year of wildlife habitat in the United States (Babbitt 1999), and more broadly are causing billion dollar losses to crop and forest production (Pimentel et al. 2005). One such invasive weed is Dalmatian toadflax, *Linaria dalmatica* (L.) Miller (Plantaginaceae), which has become abundant throughout western North America (Duncan et al. 2004).

A major effort has been made in recent years to control Dalmatian toadflax using biological control by introducing host-specific insects to North America that attack the plant (Sing et al. 2016). Biological control is one of several methods commonly used to suppress the biological impact of invasive weeds in rangelands, and in recent years, it has
gained considerable recognition in weed management largely in response to herbicide resistance (Vencill et al. 2012, Evans 2002, McFadyen 1998). Weed biological control programs attempt to re-infest invasive plants with their natural and co-evolved herbivores from their native region, with the goal of providing sustainable long-term suppression of the weed population. Biocontrol agents (usually insect herbivore specialists) can permanently reduce weed presence to below economic injury levels, while also limiting spread to new areas (DeBach and Rosen 1991, Hajek 2004). The process of biological control is generally long and laborious, but even so it is considered the most sustainable and cheapest method of long-term control of noxious weeds when it is successful, and this is especially true in rangelands (Louda and Masters 1993, Evans 2002, Quimby et al. 1991).

Although plant-feeding insects may be found to be suitable biological control agents as judged by screening tests prior to release (e.g., testing for host specificity, climate matching in the new range, and severity of feeding damage to the plant (Harris and Zwölfer 1968), effective weed control by biocontrol insects is largely unpredictable after release (Julien et al. 1984). It is often difficult to determine why some biocontrol agents fail to provide effective control, but factors likely include the inability of insects to establish and build to impactful populations because of unfavorable climates, and poor synchronization with the host plant (Julien 1997). With rapidly spreading weed populations resulting from anthropogenic distribution and climate change, it is important to understand the mechanisms of successful and unsuccessful biocontrol to make better informed management decisions in the future (Peters et al. 2014). The present study
contributes to such effort for Dalmatian toadflax by examining the biological control of the weed in northern Utah through release of the stem-mining weevil, *Mecinus janthiniformis* Toševski and Caldara (Coleoptera: Curculionidae).

**Study Organisms**

Dalmatian toadflax is an herbaceous perennial weed introduced by the late 1800s to Canada from Eurasia as a desirable ornamental plant (Sing et. al 2016, Robocker 1974). Individual plants have tall erect stems and bear alternating yellow flowers which may be arranged in a loose rosette at the base of the plant (Sing et al. 2016). Dalmatian toadflax is economically important due to its competitive nature in range and wildlands, avoidance by livestock, and resistance to chemical and mechanical control methods (Vujnovic and Wei 1997). Indeed, Dalmatian toadflax has spread throughout much of northwestern North America, and is currently spreading to southwest U.S. states including areas in Utah, Arizona, Colorado, and New Mexico (Phillips and Crisp 2001, Zouhar 2003, USDA 2014, Dodge et al. 2008, Ashigh et al. 2010). It is highly competitive to native and forage plants in mountain grasslands, valleys, and foothills as well as in sagebrush ecosystems between 1,300 and 3,100 meters in elevation (Pyke 2000, Zouhar 2003, Park 2013).

Recently identified as distinct from the yellow toadflax feeding weevil, *Mecinus janthinus* Germar, *Mecinus janthiniformis* is native to Eurasia, and was first introduced to British Columbia, Canada in 1991 as a biocontrol agent for Dalmatian toadflax (Jeanneret and Schroeder 1992, Toševski et al. 2011, De Clerck-Floate and Harris 2002). Previous research on the phenology of the weevil in the northwestern U.S. and Canada revealed
that adults emerge from overwintering sites in early-May and begin mating until early-June (McClay and De Clerck-Floate 2002). Initial observations made in Utah found evidence that the species is protandrus, with males emerging from overwintering sites considerably before females (*personal observation*). Within one week after oviposition inside stems, stem-mining larvae hatch and begin creating 1-3 cm-long mines in which they pupate and overwinter as adults until exiting the following spring (Jeanneret and Schroeder 1992, McClay and De Clerck-Floate 2002). Because calendar-based descriptions of phenology from northern regions of North America have limited application to Utah sites experiencing warmer winters, one objective of this study is to model weevil phenology (including as applies to protandry) using degree-day accumulations.

**Degree-Day Modeling and Protandry**

The metabolic function of insects and other invertebrates is highly regulated by surrounding temperatures (Allen 1976). To progress through different stages within their life cycles, insects require specific accumulations of heat, a phenomenon that can be studied using physiological time as measured by degree-days (Zalom et al. 1983). The simplest and earliest degree-day model was developed using daily mean temperatures to measure heat accumulation between high and low developmental thresholds (Arnold 1960). More recent models have added adjustments to further account for variability in temperature (University of California IPM). Degree-day models are important components of insect pest management because they provide predictability regarding insect phenology which can be applied between years and among many geographic
ranges of the insect’s distribution. Alternative models using calendar dating do not account for seasonal and climatic variability in temperature, often resulting in inaccurate results that jeopardize the success of insect biological control programs (Wilson et al. 1983).

In many animal taxa, the phenology of males and females within a species may differ, producing shifted sex ratios at specific times during a season. Protandry, the earlier arrival of males to breeding areas, is a common example of sex-biased timing in insects that can result from differences between male and female phenology (Thornhill and Alcock 1983). Several hypotheses have been proposed to explain either directly or indirectly the evolution of protandry in animal taxa, but the adaptive significance of protandry is not fully understood (Morbey and Ydenberg 2001).

Initial observations of *M. janthiniformis* in Utah indicate that male weevils emerge earlier in the spring than females, an example of insect protandry. Few previous studies have examined differences in male and female abundance in field censuses of *M. janthiniformis* on given dates, and none have described protandry within a season (Carney 2003, Carney et al. 2004). Phenological patterns in herbivorous insects are the result from many factors, including host plant biology that inadvertently regulates phenology to synchronize with plant development (e.g. flowering) and plant characteristics (e.g., height, density, etc.) needed for survival (van Asch and Visser 2007, Price 1991); however, the adaptive significance of protandry is not clear in the case of *M. janthiniformis*. Phenology in this species may be associated with adult female preference for particular stems, as indicated by previous research, leading to life cycle synchrony
between *M. janthiniformis* and Dalmatian toadflax (Jeanneret and Schroeder 1991, Carney 2003, Saner et al. 1994). This possibility warrants further investigation.

**Current Status of Dalmatian Toadflax Biological Control**

Effective biocontrol of Dalmatian toadflax by *M. janthiniformis* weevils is likely due to a synergistic effect of internal larval mining and external adult feeding on stems causing damage and potential death (De Clerck-Floate and Harris 2002, Carney 2003). *Mecinus janthiniformis* is thus capable of providing effective biocontrol of Dalmatian toadflax in sites where it can successfully establish and reach impact populations (Jeanneret and Schroeder 1992). To date, successful biocontrol has been widely observed in northern or mountainous climates of British Columbia and the American northwest, including higher elevation sites in Utah, but establishment is slow and unpredictable in regions characterized by hot/dry summers, milder winters, and lower elevations such as the Colorado Front Range and study sites in northern Utah, which are located in the weevil’s current southern range (Van Hezewijk et al. 2010, Schat et al. 2011, Jamieson et al. 2012, Sing et al. 2008, A. Mendenhall, USDA-APHIS, personal communication).

Weevils were initially introduced to these lower-elevation study sites in Utah between 2005 and 2006 (A. Mendenhall, USDA-APHIS, personal communication) and no evidence of weed suppression occurred until 2015. It is not known if these sites and others in the weevil’s southern range have environmental effects on weevil survival and phenology such that weed suppression is inhibited.
Objectives

This study addressed the varied biological control success of the noxious weed Dalmatian toadflax by the stem-boring weevil, *Mecinus janthiniformis*, in North America by evaluating insect phenology and compatibility with the host at sites in northern Utah where weed suppression has been slow to occur. The primary objectives of this study were to: 1) use degree-day models to describe the weevil’s phenology at these sites for the general population, and for each sex, 2) determine the degree to which the insect’s phenology may be synchronized with the development of the host plant such as to promote effective control, and 3) assess the overall capability of *Mecinus janthiniformis* to provide effective control at these sites in northern Utah in comparison to sites experiencing successful control. The results of this study are meant to help practitioners of Dalmatian toadflax biocontrol make decisions regarding weevil assessment, and to provide a foundation for research on the development of new biocontrol programs in the future.

Methods and Materials

Populations of Dalmatian toadflax and *M. janthiniformis* were sampled at two sites (Lake Point and Pine Canyon) in 2014-2016 in Tooele County, Utah. Weevils were sampled at a third site, Kennecott, in Tooele County only in 2014. All sites are located in the southern region of *M. janthiniformis*’ current distribution in North America and at an elevation between 1300-1500 meters above sea level. The weevil had been released at these sites in the decade prior to the present study but had been slow to establish while
the weed remained at high density (roughly 2-4 stems taller than 15cm present in randomly sampled .1m$^2$ rings between sites). Dalmatian toadflax occurred at these sites amongst a mixture of grasses (including introduced cheatgrass, bulbous bluegrass and intermediate wheatgrass as well as native species), herbaceous forbs (including western salsify, wand mullein, globe mallow, sego lily, alfalfa, and gumweed) and scattered shrubs (sagebrush, rabbitbrush, and snakeweed). The three sites were located within 12 km of each other and have experienced past disturbance due to construction and heavy grazing, thus providing an entry for Dalmatian toadflax establishment.

**Population Census**

Population censuses of *M. janthiniformis* and Dalmatian toadflax were conducted throughout the growing seasons of 2014-2015 at two sites. At the first site, Lake Point (a release site near the town of Lake Point, Utah: 40°41′56.6″N 112°15′18.9″W), repeated censuses were taken at a single location roughly 150 m in diameter and marked by a single post. At the second site, Pine Canyon (open land managed by the BLM: 40°34′25.9″N 112°14′56.5″W), censuses were taken on each occasion at 4 locations (marked by four posts) along a linear, 500 meter transect. Both Pine Canyon and Lake Point are near well-used roads, and have become heavily infested with Dalmatian toadflax. Several periodic releases of *M. janthiniformis* were made at both sites, starting with 500 weevils initially in 2005 at Lake Point and in 2006 at Pine Canyon (A. Mendenhall, USDA-APHIS, personal communication). Two additional releases of 250 weevils were made at Pine Canyon (one in 2007 and the other in 2008) while only one additional release of 250 weevils was made at Pine Canyon in 2008.
Censuses for weevil abundance were taken on 1-2 days per week from 10 April to 8 June in 2014 and on 1-4 days per week from 21 March to 29 June in 2015 at both sites. To estimate population density in 2014, 40 individual stems 25 cm or more in height were examined on each censusing occasion (at one area at Lake Point and four areas at Pine Canyon) for the presence of feeding damage and the number of adult weevils found anywhere on the plant. Plant characteristics such as height, flowering and/or budding activity were recorded concurrently. Stem height is closely correlated with stem diameter (e.g., \( r = 0.71, P < 0.0001 \), for height vs. stem diameter, as measured at the base of the plant for a random sample of \( N=200 \) stems from Pine Canyon on 21 May 2015), and hence only height was measured during censusing to estimate the overall size of the plant. Stems were picked blindly (i.e., haphazardly without bias as to height, flowering condition, or other aspects) as the closest stems to sampling points spaced at two-meter intervals along transects radiating out no more than 20 meters from the release posts.

Sampling efforts for the population census in 2015 were similar to those of the previous year but included a larger sample size and additional measurements. On each sampling occasion, 100 stems were sampled at Lake Point and 50 stems were sampled at each post at Pine Canyon for a total of 200 stems. Estimates for weevil abundance in 2015 were conducted similarly to 2014, but stems \( \geq 15 \) cm were included in the census, and individual weevils were sexed as encountered, using 10X-23 mm Doublet hand lenses. If high numbers of weevils were encountered on a stem (usually \( > 5 \) per plant), individuals were collected and contained in vials before sexing and returned to the plant
after sexing. In addition to basic plant characteristics measured in 2014, stems were also measured for basal stem diameter in 2015.

**Collecting and Sexing Weevil Adults**

Two unmarked sites were selected to make mass collections of *M. janthiniformis* adults for determining the sex ratio of individuals present at different times in 2014. These sites were selected for collections due to the high weevil densities present at the sites, and due to concern that collection at the sites selected for population censusing (as described above) might interfere with those census efforts. The first site, Kennecott (southeast from the Kennecott Smokestack: 40°44′52.3″N 112°14′01.2″W) is a north facing slope located on private land. The second site was at Lake Point (100 m due south from the marked post of Lake Point: 40°41′53.1″N 112°15′18.2″W). This site is west facing, relatively flat, and owned privately. Collections in 2015 were exclusively made at the Lake Point site in 2015.

Sexing of adult weevils was conducted in the field or in the lab using rostral and profemural characteristics described by Schat et al. (2007) and Carney et al. (2004), respectively. Sexing was accomplished using 10X-23mm Doublet hand lenses in the field and dissecting microscopes in the laboratory.

Collections took place 1-3 times per week between 10 April and 30 June in 2014 and 1-4 times per week between 29 Mar and 29 June in 2015. Weevils at both sites were collected within an approximately 30 m² area. The time spent collecting ranged from 10-60 minutes depending on the varying abundance of weevils at different times of the season and resulted in collections of up to 200 adults. Individuals were collected
indiscriminately on whole stems blindly selected and were stored in collecting vials until
sexing. Early and late in the season of 2014, collections of weevils were sexed in the field
using hand lenses. In mid-season when weevil density was high, 50-100 individuals were
brought to the lab to be sexed. In 2015 a maximum of 100 weevils were collected and
sexed in the lab. Individuals brought to the lab for sexing were often frozen until sexing
could be conducted.

**Plant Density Census**

After taking the population census (as described above), plant density was
measured at Lake Point and Pine Canyon for both the 2014 and 2015 seasons by placing
0.1 m² rings along transects radiating from the center post at each site (one post at Lake
Point and four posts at Pine Canyon). Data recorded for each ring included the number of
stems above 15 cm in height, height of the tallest stem, number of flowering stems, and
the total number of flowers and seed heads per ring. Rings were placed in front of the
forward foot upon walking approximately 4 meters along each transect. In 2014, 8
sampling transects were chosen at each center post running in each of the cardinal and
sub-cardinal directions. Five ring samples were taken every four meters along each
transect resulting in 40 ring samples total for each post. Two additional transects, also
running from the central post, were added to the census in July 2015, resulting in 50 ring
samples for each post. Data taken before this date in 2015 was recorded along the
standard 8 transects.
Flowering Stem Census

Because flowering stems were rarely encountered in the 2015 population census, additional sampling was conducted on June 8 at both sites to target flowering stems for inclusion in the data set. Any flowering stem within the 20-meter sampling radius of each post was selected and measured in the same way as in the population census described above. If two or more flowering stems were judged to be from the same individual plant, only the closest stem to the post was included in the sample. Because stems for this sample were not selected blindly, they are not representative of flowering rates for the population and were thus analyzed separately from stems in the general population census.

On May 19, 2016 at Pine Canyon, sampling was conducted to compare the number of weevils present on flowering and non-flowering stems of a similar height. In this sample, 40 non-flowering stems were selected blindly at 5m intervals along a linear transect and were measured for height and the number of male and female adults present. The nearest flowering stem of a similar height was selected for each non-flowering stem and adult weevils were counted similarly, resulting in a total of 80 stems examined. Because a large portion of adults were located on the top portion of each stem (55% of adults), the census included whether each adult was located on the top 10 cm or the rest of the plant. When adults were found in physical contact with each other on the host plant, they were recorded as engaged in copulation (“mating”) or in mate guarding (“guarding”; i.e., with the male on top of the female, presumably guarding her from other males (Alcock 1994)).
Degree-Day Modeling

Archival weather data used in degree-day models was taken from the Utah Climate Center Tooele weather station between 1 January to 31 August for each year (station ID: USC00428771). A single sign model was generated using daily minimum and maximum temperatures over a critical temperature of 8.9°C and below a horizontal cutoff at 28.7°C (University of California IPM, Jones et al. 2015, Dixon et al. 2009). This model yielded very similar patterns to a simpler max-min model including no horizontal cutoff, so both models can be used, but results from only the single sine model are presented.

Analyses

Statistical analyses were conducted using SAS (SAS 9.3 Institute 2009). Because population dynamics of *M. janthiniformis* and Dalmatian toadflax were similar between the four posts at Pine Canyon in 2014 and 2015, data collected at each of the four posts at Pine Canyon on each sampling date for 2014 and 2015 were pooled prior to all analyses.

To determine the effects of site (Lake Point versus Pine Canyon) and year (2014 versus 2015) on the mean number of *M. janthiniformis* adults found on a Dalmatian toadflax stem on the day of peak weevil density, a two-way ANOVA using PROC GLM in SAS was conducted.

Sexing data at the two sites in 2014 were combined for analysis following an initial $\chi^2$ analysis showing that the two sites did not differ significantly in sex ratio patterns over the season. To compare the numbers of males and females in collections of
adults made at successive periods throughout the 2014 and 2015 seasons, \( \chi^2 \) analyses were performed for (1) Lake Point and Kennecott combined in 2014, and (2) Lake Point in 2015. Sampling dates were grouped into six periods of time (early March through early April, late April, and early and late May and June) for analysis. In addition, linear regressions were conducted on the percentage of weevil adults that were males, as a function of the degree-day accumulation or calendar date for collections throughout the season in 2014 (again with data combined from the two sites) and 2015.

The highly significant equation obtained from regressing percentage males against degree-day accumulation in 2014 was used to estimate the percentages and absolute numbers of adult males and females per host plant stem in population censuses in 2014 (when adults were not distinguished by sex during censuses). Similar estimations were not necessary to measure percentages and absolute numbers of males and females per stem in 2015 when all individuals encountered in the population census were sexed.

A single paired t-test was used to compare degree-day accumulations for males versus females at peak density. The number degree-days accumulated for each sex was compared for each site in both years, resulting in 4 pairs in the analysis (two dates in 2014 and 2015 for each site).

Additional paired t-tests were used to compare four plant characteristics: (1) height of the tallest stem per 0.1 m\(^2\) ring, (2) density in 0.1 m\(^2\) rings, (3) the average number of flowers per stem in population census, and (4) the mean height of stems in population census as measured at comparable times (i.e., similar dates) during 2014 and
2015. Data for each individual test included measurements for each of the 8 sampling dates in 2014 and the closest corresponding date in 2015.

Linear regressions were used to test the effect of stem height on the total number of adults present (males and females combined) for 2014-2016 data, as well as the effect on individual sexes for data in 2015 and 2016. Regressions included the effect on weevil presence on the whole stem (2014 and 2015 data) as well as presence on the top 10 cm (2016 data only). Dates for all analyses (21 May 2014, 21-22 May 2015, 8 June 2015, and 19 May 2016) were selected when stem height was variable and high numbers of weevils were present.

One-way ANOVAs were conducted for data collected on 21 May 2014 at Lake Point and at Pine Canyon to compare the number of adults present on flowering and non-flowering stems, but sample sizes of flowering stems were low. With a larger sample size of flowering stems in 2015, additional one-way ANOVAs were conducted on data collected at Pine Canyon and Lake Point on 8 June 2015 to compare the number of total adults present for the sexes combined and for each sex individually on flowering versus non-flowering stems. Another one-way ANOVA was conducted to compare the average height of flowering versus non-flowering stems at Lake Point and Pine Canyon on 8 June 2015.

To determine any interactive effect of stem height and flowering on male or female presence on the whole stem, an ANCOVA was conducted on the flowering and non-flowering stem data collected at Pine Canyon on May 19, 2016, using height as the covariate. Additionally, a similar ANCOVA was conducted for the same 2016 data, but
only included the number of total adults (male and female combined) and the number of females present on the top 10 cm.

To determine the effect of stem height and flowering on the number of mating pairs observed on the whole stem and the top 10 cm, ANCOVA was conducted for data collected at Pine Canyon on 19 May 2016.

**Results**

**Seasonal Patterns of Weevil Abundance**

Patterns of abundance were generally consistent in both years at both sites, with weevils emerging at low densities by mid-April, increasing to peak abundance in May, and decreasing in numbers thereafter until disappearing altogether by the end of June (Fig. 2.1). Although trends were similar, there were some differences in phenology observed between Lake Point and Pine Canyon within a year, and between years for each site.

In 2014, sites differed in phenology in that weevil abundance at Pine Canyon remained high in late May even as weevil abundance was decreasing rapidly at Lake Point (Fig. 2.1). Also, the population decline beginning in late May 2014 was much more gradual at Pine Canyon than at Lake Point. Seasonal patterns of weevil abundance at the two sites in 2015 were very similar even as density fluctuated much more day-to-day than as observed in 2014 (Fig. 2.1).

Although seasonal patterns in each year were broadly similar at individual sites, some differences between years occurred at both sites (Fig. 2.1). Weevil numbers on the
host plant rose much more rapidly in late April in 2015 than in 2014, and remained at high densities for a longer period of time (i.e., throughout May) than in 2014. Following the occurrence of peak population density in late May, the decline in adult abundance during June was more gradual in 2015 than in 2014, especially at Lake Point (Fig. 2.1).

Peak weevil abundance was recorded on 21 May and 27 May in 2014, and on 29 May and 18 May in 2015, at Lake Point and Pine Canyon, respectively. The number of weevils observed per stem during peak abundance was significantly higher at both sites in 2015 than in 2014 and significantly higher at Lake Point than at Pine Canyon for both years (Table 2.1). The difference in density at peak abundance between the two sites was especially marked in 2014, however, and was much less evident in 2015 although both sites experienced significant increases (Fig. 2.1).

The more rapid increase in weevil abundance during late April in 2015 than in 2014 was associated with warmer early spring conditions in 2015 and correspondingly with more rapid gain in degree-days during this period than in 2014 (Fig 2.2). Similarly, the more rapid drop in *M. janthiniformis* numbers in late May in 2014 than in 2015 was associated with more rapid gain in degree-days during this period in 2014. Despite differences in the seasonal timing of heat gain in 2014 and 2015, overall heat gain in the two years was very similar from January to the end of June when *M. janthiniformis* adults no longer were found on the stems (Fig. 2.2).

**Evidence of Protandry: Mass Sexing**

In both 2014 and 2015, random samples of *M. janthiniformis* adults collected throughout the spring and early summer revealed a strong seasonal pattern in sex ratio.
Early in each spring almost all individuals found on the host plant were males, whereas most individuals after the population had peaked were females. Differences in the relative abundances of males and females over time were highly significant, with the period of approximately equal numbers of both sexes occurring in early June 2014 and late May 2015 (Table 2.2). For all collections combined (i.e., from March through June), many more males than females occurred on the host plants in both 2014 and 2015.

When the percentage of males (reflecting the sex ratio) was regressed against of degree-day accumulation, a significantly negative slope was obtained in each year (Fig 2.3). In both years there was a steady decrease in the percentage of males as degree-days accumulated and females began to emerge (Fig 2.3; linear regressions of male percentage by degree-day accumulation: $F_{1,18} = 226.2$, $P < 0.001$, $R^2 = 0.93$ for 2014 and $F_{1,35} = 446.8$, $P < 0.001$, $R^2 = 0.93$ for 2015). The percentage of males similarly decreased with calendar date ($F_{1,18} = 168.2$, $P < 0.001$, $R^2 = 0.90$ for 2014, and $F_{1,35} = 264.0$, $P < 0.001$, $R^2 = 0.883$ for 2015). From the equations obtained in linear regressions, the sex ratio (males: females) can be estimated at 1:1 (50% male) at 463 degree-days in 2014 and at 411 degree-days in 2015 for male percentage by degree-day accumulation. For the regression of male percentage by calendar date, a 1:1 sex ratio was found on Julian day of year 151 (31 May) in 2014 and on day 144 (24 May) in 2015. These estimates are consistent with the results in Table 2.2.

**Evidence of Protandry: Stem Census**

When examined by degree-day accumulation, *M. janthiniformis* populations rapidly increased in the early spring once adults began becoming active. This is apparent
for each sex across sites and years (Fig. 2.4). Males emerged earliest onto the host plant and reached peak population density at considerably fewer degree-day accumulations than females in both 2014 and 2015 (paired t-test comparison of degree-day accumulations for males versus females at peak density for both sites in both years: \( t_3 = 5.65, P = 0.011 \)). The timing of peak abundance in response to degree-day accumulation was roughly consistent across sites and years for both sexes, and especially so for males (Fig. 2.4). Although their numbers remained relatively high for a longer period in 2014 than in 2015, males first attained peak numbers at very similar degree-days at both sites in each year (with the peak occurring between 255-270 degree-days). Females also peaked in abundance at fairly consistent accumulations of degree-days at both sites in both years, but with more variability than observed for males (with the peak occurring between 307-373 degree-days). This variability in peak female abundance between years is most pronounced at Lake Point, with females peaking in number after considerably more degree-days had accumulated in 2015 than in 2014. In all cases, numbers of both males and females declined with continuing degree-day accumulation more gradually after peaking than they had increased prior to peaking (Fig. 2.4).

In 2014, degree-day accumulation resulted in males peaking in abundance on the host plant several days before females at both sites (Fig. 2.5). In 2015, however, a more protracted accumulation of degree-days as males and females were increasing in numbers on the host plants led to a considerable difference in the date when the two sexes peaked in abundance. In 2015, abundance of males peaked twenty-four days before females at Lake Point and eighteen days before females at Pine Canyon, with both peaks occurring
while female abundance was still quite low. Although peak female abundance did not coincide with peak male abundance in 2015, relatively high numbers of male individuals persisted on the host plant when females became most abundant (Fig. 2.5).

The difference between years in the number of days occurring between peak male and female abundance arose largely from males reaching peak abundances on earlier dates at both sites in 2015 than in 2014 (Fig. 2.5). Females peaked in abundance on similar dates at Pine Canyon in 2014 and 2015, and a little later in the spring at Lake Point in 2015 than in 2014 (which led to greatest separation in dates of peak male and female abundance occurring at Lake Point in 2015).

The patterns of abundance of the two sexes at Lake Point and Pine Canyon in 2014 and 2015 are consistent with results from collection sites in both years. Males greatly outnumbered or equaled females in their abundance throughout the majority of the season (Fig. 2.5) while females outnumbered males by a wide margin only at the end of the season, when populations were declining (i.e., late-June; Table 2.1).

**Host Plant and Weevil Phenology: Successful Synchrony and Effective Control**

Toadflax stems grew rapidly during the spring in both years. By late May to early June, stems had reached their maximum height and density after which little additional growth was observed (Fig 2.6). The first flush of flowering also occurred in late May to early June, but the sporadic nature of flowering among individuals resulted in unclear trends in flowering intensity throughout the remainder of the season. Peak numbers of female weevils were found on the host plants in late May and early June as the stems
reached maximum height and density, and as the stems began to flower widely (Fig 2.6; arrows).

The largest stems at both sites, as recorded in ring sample censusing, were significantly shorter in 2015 than in 2014 (Fig 2.6A; paired t-test for mean height of tallest stems on the same or nearly the same sampling dates between 2014 and 2015: \( t_7 = 12.25, P < 0.0001 \) for Lake Point and \( t_7 = 5.02, P = 0.002 \) for Pine Canyon). When including all stem heights surveyed during the population censuses, results similarly reflected that the stems were significantly shorter in 2015 than in 2014 (paired t-test for average stem height on corresponding dates between 2014 and 2015: \( t_{20} = 22.23, P < 0.0001 \) at Lake Point and \( t_{20} = 16.79, P < 0.0001 \) at Pine Canyon). The average height of the largest stems occurring in all ring samples was reduced from 61.78 ± 2.45 cm in 2014 to 36.32 ± 1.47 cm in 2015 at Lake Point and from 91 ± 7.65 cm to 50.09 ± 1.88 cm at Pine Canyon. For the dates on which female weevils reached peak abundance, this height was similarly reduced from 39.56 ± 1.75 cm to 21.76 ± 1.28 cm at Lake Point and from 41.59 ± 0.99 cm to 20.06 ± 0.72 cm at Pine Canyon between 2014 and 2015, respectively.

Similar trends emerged when comparing stem density between years at each of the two sites (Fig 2.6B; paired t-test for mean stem density per matched sampling date in 2014 and 2015: \( t_7 = 10.99, P < 0.0001 \) at Lake Point and \( t_7 = 18.05, P < 0.0001 \) at Pine Canyon). In 2014, stem density reached a peak average of 2.28 ± 0.36 stems > 15 cm per 0.1 m² at Lake Point on 19 June and 4.56 ± .3 stems > 15 cm per 0.1 m² at Pine Canyon on the same day (Fig. 2.6B). In 2015, Lake Point and Pine Canyon reached only 0.65 ±
0.22 stems per 0.1 m$^2$ on average at peak density on 26 May and 1.37 $\pm$ 0.17 stems per 0.1 m$^2$ at peak density on 29 May respectively (Fig. 2.6B). At both sites in 2015 (when sampling began early in the spring), a nearly linear increase to peak stem density occurred, with stem density increasing incrementally during March, April and May until reaching peak density. After peak density, the sites differed in seasonal trends in stem density, with the density at Pine Canyon remaining steady after reaching maximum density while stem density decreased (as mature, dried stems broke and fell over) after reaching maximum density at Lake Point (Fig. 2.6B).

In 2014 and 2015 there was little to no flowering activity during April and July at either site. Variable numbers of flowers per stem occurred with several sporadic flushes during May and June at both sites in both years (Fig. 2.6C). The average numbers of flowers observed on stems in 2014 reached a peak average of 1.2 $\pm$ 0.45 flowers on 16 May and 2.36 $\pm$ 0.34 flowers on 30 June at Lake Point and Pine Canyon, respectively. Although peak flowering per stem occurred later in the season at Pine Canyon than at Lake Point in both years, there was a substantial decrease in flowering activity at both sites from 2014 to 2015 (paired t test for mean number of flowers per matched sampling dates in 2014 and 2015: $t_{20} = 3.2$, $P = 0.004$ for Lake Point and $t_{20} = 3.89$, $P = 0.0009$) with peak numbers of flowers per stem declining to 0.18 $\pm$ 0.11 per stem at Lake Point on 18 May and 0.1 $\pm$ .04 per stem at Pine Canyon on 17 August respectively in 2015.
Weevil Response to Stem Height and Flowering Condition

A positive correlation (significant on all occasions with the exception of Pine Canyon in May 2014) occurred between the number of adults observed and stem height of individual stems measured on individual occasions in May or June of each of three years (2014-2016) at several sites (Figs. 2.7 and 2.8). This was true for males and females when comparing the sexes on 21-22 May and on 8 June of 2015 (when adults were sexed individually during the stem census), but the relationship was stronger for females (Figs. 2.9 and 2.10).

Few flowering stems were encountered during 21-22 May 2015 censuses, and no significant difference was observed between the numbers of adults present on these versus non-flowering stems at either site ($F_{1,38} = 0.56$, $P = 0.46$ at Lake Point and $F_{1,158} = 0.6$, $P = 0.44$ at Pine Canyon, with data including 8 flowering stems at each site). When greater sample sizes were obtained by inspecting additional, randomly selected flowering stems on 8 June 2015 (i.e., including stems examined beyond those included in the census), flowering stems were found to have significantly more adult weevils than did non-flowering stems, and this was consistent between the sexes (Fig. 2.11). Flowering stems on average were also significantly taller ($45.88 \pm 1.67$ cm) than non-flowering stems ($21.73 \pm 0.47$ cm) on average at both sites on 8 June 2015 (Lake Point: $F_{1,128} = 113.85$, $P < 0.0001$ and Pine Canyon: $F_{1,237} = 255.41$, $P < 0.0001$).

When examining flowering and non-flowering stems that varied similarly to each other over a large range of stem heights on 19 May 2016 (i.e., flowering and non-flowering stems of similar heights were censused at random locations along transects), an
ANCOVA yielded a significant positive effect of stem height on the number of individuals of both males and females occurring on the entire stem (Table 2.3), and on the terminal 10 cm of each stem where roughly 55% of males and females were located (Table 2.4 and Fig. 2.12). When controlling for the effect of stem height in the ANCOVA, there was no significant effect of stem reproductive condition (flowering versus non-flowering) or interaction between the variables on the number of weevil adults of either sex on the entire stem, or on the terminal 10 cm (Tables 2.3 and 2.4).

Approximately 20% of the population was observed to exhibit mating behavior (“mating” and “guarding”) at Pine Canyon on 19 May 2016. The number of “mating” plus “guarding” pairs (average of 0.34 ± 0.07 pairs per stem) distributed among stems varied similarly to the general population above, with height being the most consistent influencing factor determining the number of mating pairs on an individual stem (Table 2.5). When stem height varied similarly between flowering and non-flowering stems in the 2016 sample, we observed a significant effect of height on the number of mating pairs on the whole stem, and on the top 10 cm, regardless of reproductive condition (Fig. 2.13). Approximately 52% of pairs were located on the top 10 cm of each stem.

**Discussion**

To understand the potential for widespread biological control of the noxious weed Dalmatian toadflax by the stem-mining weevil *M. janthiniformis* in North America, this insect-plant interaction must be studied at multiple locations where the biocontrol agent has been released. The present study focuses on two release sites in northern Utah as
studied especially in 2014 and 2015, and the results can be used to address the following questions concerning the weevil and the weed: (1) Does degree day modeling of weevil phenology give consistent results between the two study years even as differences may occur in calendar date timing between the two years? (2) Do males and females differ in their patterns of spring emergence and seasonal appearance on the host plant, such that degree-day modeling can give even more useful results when the two sexes are considered individually? (3) Is the phenology of the weevil synchronized with the phenology of its host plant? And (4) is the matching of the weevil’s and the host plant’s phenology in the arid Utah climate (i.e., in the southern range of the insect-plant interaction) suitable to promote effective biological control? These questions are each considered in turn below.

Models of Phenology

*Mecinus janthiniformis* adults in their native region (formerly Yugoslavia) generally emerge from overwintered toadflax stems onto new shoots in May, oviposit from late May to mid-July, and die shortly thereafter (Jeanneret and Schroeder 1992). In North America, observations of adult weevils on host plants can occur by early April, depending on early spring weather conditions, and the weevils remain active on the host plants until early July (Sing et al. 2016). Weevils in northern Utah conform to these observations, with the only slight difference being an earlier arrival on the host plant (a few adults emerged in late-March in the present study), and this is likely due to warmer spring conditions in Utah than in more northern locations.
In general, weevil phenology is consistent between the two sites studied each year and between the 2014 and 2015 growing seasons. Temperature variation between the two study years can largely account for calendar-date variation in timing of emergence in April to mid-May and rate of population die off late in the season. These variations are largely resolved in the temperature based degree-day models, supporting the utility of using degree-day modeling to generate better fitting models of phenology (Zalom et al. 1983, Herms 2004, Sridhar and Reddy 2013). While degree-day modeling indeed reduces variation in our models, there are still small, but clear differences in *M. janthiniformis* phenology between sites and between years in this study.

In 2014 we observed calendar-based differences in weevil phenology between sites, with weevils reaching peak abundance at Pine Canyon while they were on the decline at Lake Point. Although one would expect similar weather conditions to occur at the nearby sites, there may be small microclimatic differences between Pine Canyon and Lake Point, leading to the variation in phenology between these sites in 2014. In 2015, however, phenology was very consistent between the sites, so there may be other variables not measured, such as precipitation, which may have influenced phenology in 2014. Because data for degree-day models only included temperature from a central station in Tooele, it is not clear whether these small phenological differences in 2014 can be accounted for by microclimate differences between Lake Point and Pine Canyon.

Phenology was also largely consistent between 2014 and 2015, but there were some variations between the years that occurred at both sites. First, at both sites in 2015 sharper fluctuations in population density occurred between sampling dates than at these
sites in 2014. The jagged lines for 2015 in Fig. 2.1 reflecting these fluctuations are likely the result of the periodic heavy rains observed throughout May in 2015. During inclement weather such as occurred in the spring of 2015, weevils may release from the host plant upon impact by rain and drop to the ground to seek refuge (weevils frequently respond to disturbances in this manner, and the behavior is called thanatosis or “death-feigning” (Kuriwada et al. 2010). Additional adult weevils may be knocked loose and blown from stems by the wind with inclement weather. Once on the ground, weevils are very difficult to observe. Second, weevils in 2015 remained at high densities for a longer period of time than in 2014. This is largely due to males reaching their peak abundance before females in 2015, leading to a rounder curve in Fig. 2.1 and a less defined date of peak density for the whole population.

Overall, the results show spatial and temporal consistency especially in degree-day accumulation but also in calendar-based descriptions of weevil phenology. Although the degree-day models account for temperature variations between years, the results revealed slight differences in phenology between sites and between years that may be due to sampling error, or potentially other climatic variables not included in the models.

**Male and Female Phenology:**
**Protandry and Management Implications**

This study demonstrates that males and females differ in phenology, with protandry being a pattern consistent at all sites in each year, but especially pronounced at sites in 2015. Because males and females differ in their patterns of spring emergence and seasonal appearance on the host plant, degree-day modeling can give even more useful
results when the two sexes are considered individually. Protandry within a growing season has not been described previously for *M. janthiniformis* adults; however, a study by Carney (2003) found skewed sex ratios of adult weevils that were collected in the latter half of June in British Columbia, with weevils being 59-68% female during this time in the season. These findings were largely supported in this study, with sex ratios being female skewed beginning in early June at sites in Utah. Results from the present study, and the study by Carney (2003), suggests that protandry of *M. janthiniformis* may be common for the species at sites spanning North America.

This discovery that protandry occurs in *M. janthiniformis* poses interesting questions about its biological significance. Protandry is a common phenomenon in insects as described by Thornhill and Alcock (1983), but the adaptive significance is largely unknown for specific species (Morbey and Ydenberg 2001). Several hypotheses have been developed to apply broadly in helping to explain protandry in insects, including the influence of sexual selection (Lehmann 2012, Morbey et al. 2012, Morbey and Ydenberg 2001). Because males were observed in the present study to outnumber females during most of the season, it is plausible that there is some form of female choice for mates and thus male-male competition. One can speculate that early emerging males of *M. janthiniformis* may benefit under such a competitive scenario for several reasons, as in “rank advantage hypotheses” or “mate opportunity hypotheses” (Morbey and Ydenberg 2001). These hypotheses postulate a fitness benefit for early emerging males by acquiring preferred territory or maximizing the likelihood of encountering virgin females.
Protandry may pose challenges for biological control management practices if collections of source populations occur early in the season, when the majority of individuals are male (Hemipel and Lundgren 2000). Because the sexes of *M. janthiniformis* differ in their phenology, it is beneficial to consider them individually when determining the best time to collect them for redistribution, e.g., when an optimal sex ratio is achieved. Generally, optimal sex ratios range from 1:1 to female biased, and models that accurately predict when field populations are at optimal sex ratio are essential in promoting the success of protandrous biological control agents (Tabadkani et al. 2013). In this study, adult *M. janthiniformis* individuals had a sex ratio of roughly 1:1 between 350-470 degree-days, or from mid-May to early-June. Given that densities begin to decline in June, mid to late-May would be the recommended time during a season to collect adults for redistribution.

It is important to note that although males and females did not coincide with each other in the timing of peak abundance in 2015, males still occurred in high density when females reached peak abundance. This likely ensured successful mating, but if not, the selection pressure could act to shift male phenology to more closely align with female phenology in the future.

**Potential Drivers of Weevil Phenology:**
**Ties to the Host Plant?**

Overall, very little work has been done on the physiology of diapause and reproduction of *M. janthiniformis*, so little is known about the underlying mechanisms of phenology. While the phenology of males may be driven by response to female
phenology as the result of sexual selection, the timing of female emergence may be
influenced especially by the phenology of the host plant, because females utilize stems
not only for feeding but also for oviposition. Indeed, the life cycle timing of specialized
herbivorous insects is believed to be tightly associated with the phenology of their host
plants because for their growth and reproduction they require flowers, seeds, or other
plant characteristics that are available only at certain times of the season (Rehill and

In the present study, females appeared to peak in their abundance on the host
plants each year around the time that Dalmatian toadflax stems as a population reached
full maturity, i.e., when the stems reached their maximum height and density, and when
they began flowering widely. This suggests that female weevil phenology is tied to this
aspect of host plant population development, as might be reflected further by female
preference for tall and/or flowering host plant stems. Previous studies have shown that
female *M. janthiniformis* adults indeed prefer larger stems (with correspondingly large
diameters), likely due to a minimum diameter required for successful larval development
(Jeanneret and Schroeder 1992). Use of larger stems potentially may also negate the
effects of larval overcrowding (i.e., intraspecific competition) that may lead to higher
rates of mortality (discussed in Chapter 3 for adults within overwintered stems).
Additionally, larval survival is higher in flowering than non-flowering stems under
laboratory conditions, but it is not known if adults prefer flowering stems as feeding
and/or oviposition sites in the field (Saner et al. 1994, Carney 2003).
Female preference for taller stems in the field is strongly suggested by the results of the present study. As found in previous studies by Toševski et al. (2011) and Schat et al. (2011), weevil density and feeding damage was concentrated on the apical meristems of Dalmatian toadflax stems. Thus the positive correlations of female density with overall stem height observed in 2014 and 2015 were unlikely simply a reflection of more plant biomass supporting more insects. Indeed, when plants were censused in May 2016 to address the issue further, the number of females on just the top 10 cm of stem was positively correlated with overall stem height.

Similar responses to stem height were observed for males. One might speculate that taller stems could be more palatable and/or a better source of nutrition for the weevils. Alternatively, clustering of males on taller stems may reflect strong selection to be in close proximity to females with which they seek to mate. It is important to note that although these trends were significant, our models accounted for less than 50% of the variation in this data, leading to weak regressions and considerable variability.

Peak weevil abundance occurred when stems began flowering widely as well as when stems reached peak density. Analyses of adult abundance among stems on given dates provided little evidence that adults of either sex prefer flowering stems. Because it proved infeasible to distinguish oviposition scars from feeding damage due to high levels of damage, it could not be determined if female presence on flowering stems resulted in more oviposition activity. Synchrony between peak weevil numbers and stem density may be due to few new stems developing at this time of the season when the stems
already present reach maximal average height, rather than adult preference for denser ramets.

Taken together, the results of this study suggest that weevil phenology, especially for females, may be tied to the biology of the host plant such that adults peak in their numbers when stems reach maximum height, possibly due to a preference for taller stems. There is little evidence, however, that the phenology of weevils is influenced by flowering activity of the host plant.

*Mecinus janthiniformis* in Utah: Does Phenology Promote Effective Biological Control?

Gauging how well a biological control agent can synchronize with its host is critical when determining the potential for effective control. Upon initial screening, *M. janthiniformis* was shown to be synchronized well enough with Dalmatian toadflax that it was eligible for release in the United States (USDA-APHIS 1996). At many sites, *M. janthiniformis* provided effective plant suppression within several years, rendering it the most promising control agent of Dalmatian toadflax available (Sing et al. 2008, Park 2013, Goulet et al. 2013, Van Hezewijk et al. 2010, Schat et al. 2011, Carney 2003). However, at sites located in the most southern range of the plant-insect interaction, weevils have been slow to establish and effective control is unpredictable (Jamieson et al. 2012), and this includes sites in northern Utah. These areas are typically characterized by lower relative elevations and hot/dry summers. The primary concern when releasing *M. janthiniformis* to North America was overwintering mortality due to harsh winters (De
Clerck-Floate and Miller 2002, McClay and Hughes 2007) so there have been few efforts aimed to learn more about the insects in arid regions.

Initial releases of *M. janthiniformis* were made at Lake Point in 2005, with two later releases occurring through 2008. Weevils were introduced to Pine Canyon a year later, in 2006, with one additional release made in 2008 (A. Mendanhall, USDA-APHIS, personal communication). For many years, infestation rates were so low that there was little to no evidence that adults were successfully establishing (personal communication with Tooele county land managers). Many factors may be affecting *M. janthiniformis*’ ability to establish quickly in these areas, but the results of this study suggest that asynchrony with Dalmatian toadflax is not likely to be a factor in this particular case. In fact, we found that weevils tend to reach peak density at these sites when plant reserves are generally located at the apical meristems of perennial plants, where weevil feeding is highly concentrated (Toševski et al. 2011, Chapin et al. 1990, Schat et al. 2011).

Between the 2014 and 2015 seasons, significant reductions in stem height, stem density, and flowering production were recorded at both sites that were associated with a large increase in weevil density. These results are consistent with other studies testing the effect of *M. janthiniformis* activity on plants in lab and in the field (Van Hezewijk 2010, De Clerck-Floate and Harris 2002, Carney 2003, Schat et al. 2011, Jamieson et al. 2012). The results suggest that although slow to initially establish, *M. janthiniformis* may be capable of providing effective biological control of Dalmatian toadflax in southern regions. Density of larvae within stems at study sites in 2015 was on average above control thresholds described by Schat et al. (2011), but the increase in weevil density to
this level had taken about a decade to occur. It is also important to keep in mind that plants are responding also to multiple factors other than herbivory that may have influenced the rapid decline in plant vigor between two years. Thus, investigations of plant response to climatic variables typical of these regions, such as drought stress, and also biotic variables such as density dependence, should be considered (Weed and Schwarzländer 2014). Overall, land practitioners interested in long-term management without the need for urgent suppression should consider *M. janthiniformis* as a viable option for control of Dalmatian toadflax in arid climates.

**Conclusions**

The purpose of this study was to evaluate the phenology of host plant use by adults of *M. janthiniformis* within a growing season at sites in northern Utah where weevil establishment is slow to occur. Four main conclusions can be drawn from the results. First is that seasonal patterns of abundance are similar between years when expressed by degree-day accumulations, while there is more variability when phenology is assessed by calendar date. Second, when the sexes are considered individually, degree-day modeling gives very useful, predictive results that reflect that the sexes differ considerably in phenology. Third, the phenology of the weevil may be tied to that of its host plant due to preferences for tall, mature stems. Fourth, effective biological control may be promoted by the synchrony of the weevil’s phenology to that of Dalmatian toadflax, even in the hot/dry climates of southern regions. The results from this study
may be useful to practitioners of biological control when developing new programs in spreading weed populations.

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Tables and Figures

Table 2.1. Results of a two-way ANOVA on the effects of site and year on the number of *M. janthiniformis* adults observed on the host plant during peak abundance.

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<tr>
<td>Site * Year</td>
<td>1</td>
<td>16.36</td>
<td>1.98</td>
<td>0.1604</td>
</tr>
<tr>
<td>Error</td>
<td>416</td>
<td>3442.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>419</td>
<td>3947.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 2.2.** Results of a $\chi^2$ test used to compare the sex ratio over the season at Kennecott and Lake Point combined in 2014 and at Lake Point in 2015. Individual tests were conducted for females versus males as grouped into six time periods in each year.

<table>
<thead>
<tr>
<th>Dates</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P value</td>
</tr>
<tr>
<td>Mar 1 - Apr 14</td>
<td>Male: 40</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Female: 9</td>
<td></td>
</tr>
<tr>
<td>Apr 15 - Apr 30</td>
<td>Male: 113</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Female: 17</td>
<td></td>
</tr>
<tr>
<td>May 1 - May 15</td>
<td>Male: 738</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Female: 212</td>
<td></td>
</tr>
<tr>
<td>May 16 - May 31</td>
<td>Male: 499</td>
<td>0.0016</td>
</tr>
<tr>
<td></td>
<td>Female: 404</td>
<td></td>
</tr>
<tr>
<td>June 1 - June 15</td>
<td>Male: 382</td>
<td>0.1136</td>
</tr>
<tr>
<td></td>
<td>Female: 427</td>
<td></td>
</tr>
<tr>
<td>June 16 - June 30</td>
<td>Male: 247</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Female: 553</td>
<td></td>
</tr>
<tr>
<td>Totals:</td>
<td>Male: 2019</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Female: 1319</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3. Results of two ANCOVAs of reproduction (flowering vs. non-flowering stems) and height (cm) as a covariate on the number of male and female individuals found on whole stems at Pine Canyon on May 19, 2016.

<table>
<thead>
<tr>
<th>Females</th>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reproduction</td>
<td>1</td>
<td>1.29</td>
<td>0.33</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td>1</td>
<td>206.62</td>
<td>53.08</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Repro*Height</td>
<td>1</td>
<td>2.42</td>
<td>0.62</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>76</td>
<td>295.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>79</td>
<td>505.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Males</th>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reproduction</td>
<td>1</td>
<td>0.05</td>
<td>0.03</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Height</td>
<td>1</td>
<td>39.78</td>
<td>25.88</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Repro*Height</td>
<td>1</td>
<td>1.07</td>
<td>0.7</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>76</td>
<td>116.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>79</td>
<td>162.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.4. Results of two ANCOVAs of reproduction (flowering vs. non-flowering stems) and height (cm) as a covariate on the number of total individuals (males and females) or females only found on the top 10 cm of stems observed at Pine Canyon on May 19, 2016.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproduction</td>
<td>1</td>
<td>0.03</td>
<td>0.01</td>
<td>0.92</td>
</tr>
<tr>
<td>Height</td>
<td>1</td>
<td>72.69</td>
<td>25.08</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Repro*Height</td>
<td>1</td>
<td>0.83</td>
<td>0.29</td>
<td>0.59</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td>220.3</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>297.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproduction</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.92</td>
</tr>
<tr>
<td>Height</td>
<td>1</td>
<td>20.21</td>
<td>18.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Repro*Height</td>
<td>1</td>
<td>0.1</td>
<td>0.09</td>
<td>0.77</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td>84.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>104.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.5. Results of an ANCOVA of reproduction (flowering vs. non-flowering stems) and height (cm) as a covariate on the number of mating pairs observed on whole stems (top) and on the top 10 of stems (bottom) measured on 19 May 2016 at Pine Canyon.

### Whole Stems

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproduction</td>
<td>1</td>
<td>0.12</td>
<td>0.32</td>
<td>0.57</td>
</tr>
<tr>
<td>Height</td>
<td>1</td>
<td>3.91</td>
<td>10.69</td>
<td>0.002</td>
</tr>
<tr>
<td>Repro*Height</td>
<td>1</td>
<td>0.10</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td>27.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>79</td>
<td>31.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Top 10 cm

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproduction</td>
<td>1</td>
<td>0.14</td>
<td>1.05</td>
<td>0.31</td>
</tr>
<tr>
<td>Height</td>
<td>1</td>
<td>1.14</td>
<td>8.43</td>
<td>0.005</td>
</tr>
<tr>
<td>Repro*Height</td>
<td>1</td>
<td>0.16</td>
<td>1.16</td>
<td>0.29</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td>10.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>79</td>
<td>11.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2.1. Average number of *M. janthiniformis* on the host plant at two sites over the 2014 and 2015 growing seasons from March 11 (Julian day of year 70) to July 9 (Julian day of year 190).
Fig. 2.2. Degree-day accumulation (as determined using the single sine method with a critical base temperature of 8.9°C and a cutoff threshold of 28.7°C) in 2014 and 2015 as a function of Julian day of year. Day of year 200 corresponds to 8 July.
Fig. 2.3. The effect of heat accumulation on the proportion of males in field collections made throughout the 2014 and 2015 seasons. $R^2 = 0.93$ and $p < 0.001$ for 2014 and 2015.
**Fig. 2.4.** Population dynamics of male and female *M. janthiniformis* adults as a function of degree-day accumulation during 2014 and 2015. Percentage of maximum represents the abundance of individuals on each sample date divided by the maximum abundance observed at that site and year.
Fig. 2.5. Relative abundance of male and female *M. janthiniformis* adults by Julian day of year in 2014 (top) and 2015 (bottom) at Lake Point S (left) and Pine Canyon (right). Individuals per stem at 2014 sites were estimated using the regression line in Fig. 2.3.
Fig. 2.6. A comparison of stem height (A), stem density (B), and flowering activity (C) of Dalmatian toadflax at two sites over the 2014 and 2015 growing seasons. Filled arrows represent peak female abundance in 2015 while unfilled arrows represent predicted peak female abundance in 2014.
Fig. 2.7. A linear regression of the effect of stem height on the abundance of weevils on individual stems, with the sexes combined on May 21-22 of 2014 and 2015.
**2015 June**

- Lake Point
- Pine Canyon

LPS: $R^2=0.44$, $p<0.0001$

PC: $R^2=0.29$, $p<0.0001$

**2016 May**

PC: $R^2=0.43$, $p<0.0001$

**Fig. 2.8.** Linear regression of the number of *M. janthiniformis* observed on stems of varying height at two sites in June of 2015 (top) and at Pine Canyon in 2016 (bottom).
Fig. 2.9. Linear regressions of the effect of stem height on the number of male (right) and female (left) weevils present on each stem at Lake Point (circles) and Pine Canyon (triangles) in 2015. Data was collected on the 22 May 2015 for Lake Point and on 21 May 2015 for Pine Canyon.
Fig. 2.10. The effect of stem height on the numbers of males (right) or females (left) observed on stems on 8 June 2015 at Lake Point (top) and Pine Canyon (bottom).
Fig. 2.11. A histogram showing the distribution of stems on 8 June 2015 at Lake Point (top N = 33) and Pine Canyon (bottom N = 40) having 0 to 16 females (left) or 0 to 16 males (right).
**Fig. 2.12.** Linear regressions of the effect of plant height on the total number of adults (A; males and females) and females only (B) on the top 10 cm of corresponding stems. Filled and unfilled triangles represent the flowering status of a particular stem as being flowering or not flowering (i.e. “non-flower”). Data was collected on May 19, 2016 at Pine Canyon.
Fig. 2.13. The effect of stem height on the number of mating pairs present on the whole stem (A) and on the top 10 cm of each stem (B).
CHAPTER 3

OVERWINTERING SUCCESS IN UTAH OF *MECINUS JANTHINIFORMIS* ADULTS, A BIOLOGICAL CONTROL AGENT OF Dalmatian Toadflax

Abstract

Post-release monitoring of weed biological control programs is essential in determining the ability of biocontrol agents to provide effective weed control, and also to determine future prospects in biocontrol programs. Dalmatian toadflax is an invasive rangeland weed that is spreading to southern regions in the United States where the primary biocontrol agent, *M. janthiniformis*, has been slow to establish to provide effective weed control. This study investigates the mortality of *M. janthiniformis* adults during overwintering and spring emergence at sites in northern Utah where the insect has been released, but has been especially slow to establish. Stems collected and dissected at two sites during each of two years revealed low overall overwintering mortality before adult emergence from stems, and higher rates of mortality during spring and summer, especially in stems with higher densities. Adults of both sexes that survived winter, but failed to emerge from stems in the spring, appeared to be smaller than those successfully emerging, perhaps as a consequence of reduced fat reserves acquired during development. Overall, this study indicates that adult mortality during overwintering and spring emergence will likely not limit effective biocontrol of Dalmatian toadflax as it spreads to southern regions.
Introduction

In many regions throughout the world, invasive plants are reducing biodiversity, ecosystem function, and land value by outcompeting and ultimately displacing desirable vegetation. Biological control programs aim to reduce the biological impact and spread of invasive species by introducing natural enemies from their native range. When successful, this management technique is the cheapest long term method of control for invasive plants (DeBach and Rosen 1991). Such a program has been developed in North America to combat the invasive weed, Dalmatian toadflax (*Linaria dalmatica* (L.) Miller (Plantaginaceae)) using herbivorous insects including the current most promising agent, a stem-boring weevil, *Mecinus janthiniformis* Toševski and Caldera (Coleoptera: Curculionidae) (Nowierski 2004, McClay and De Clerck-Floate 2002, Jeanneret and Schroeder 1992, Toševski et al. 2011).

The degree of success in biological control is highly dependent on several factors, including the insects’ ability to establish and thrive in new areas (Stiling 1993). Original releases of *Mecinus* spp. on Dalmatian toadflax in British Columbia and the northwestern United States resulted in highly variable establishment and unreliable weed suppression (De Clerck-Floate and Harris 2002, Van Hezewijk et al. 2010, Goulet et al. 2013, Jamieson et al. 2012, Schat et al. 2011, McClay and Hughes 2007). In addition to the species identity (*M. janthinus* versus *M. janthiniformis*) of biocontrol insects released at some sites (Toševski et al. 2011, McClay and Hughes 2007), one major factor in this variable success may be the mortality rate of adults overwintering in stems. De Clerck-Floate and Miller (2002) found high mortality of overwintering adults in western Canada.
resulting from exposure to low temperatures (less than \(-28^\circ \text{C}\)) and lack of insulating snow cover. Cool temperatures in summer also may slow larval development thereby preventing individuals from pupating to the adult (overwintering) stage before winter (McClay and Hughes 2007), or undermining their ability to survive the following winter after pupation (e.g., by reducing their ability to accumulate fat reserves during the larval period). In more southerly locations in North America where \(M.\ janthiniformis\) has been released, the extent of overwintering adult mortality has not been examined closely.

Rates of overwintering adult mortality within stems, versus successful emergence from stems in the spring, were examined in the present study at sites in northern Utah where \(M.\ janthiniformis\) has been released but has been slow to establish. Patterns of mortality and emergence were compared between males and females, as males appear on new stems and foliage earlier than females in the spring and occur at higher densities on the host plant throughout much of the spring and early summer (see Chapter 2). The potential importance of body size for adult survival and emergence from the stem was examined also. The body size of an adult insect often reflects its success in obtaining nutrition during immature stages (Briegel 1990), and could be positively associated with fat reserves following pupation among overwintering adults in the case of \(M.\ janthiniformis\) (Arrese and Soulages 2010, Mirth and Riddiford 2007). The body sizes of overwintering adults were examined therefore to determine whether small individuals died at higher rates within stems than did large individuals.
Methods

Overwintered Stem Collections

Three samples of 30 overwintered stems were collected and dissected during 2014 and 2015 at several sites. Samples were collected in late-March before adult emergence from stems, in early-May when roughly half of adults had exited stems, and in late-June (2014) and early-July (2015) when all living adults had exited stems (Table 3.1). All stems were refrigerated overnight until dissections could be made the following day.

Stems were collected at two sites in 2014 including Lake Point (40°41′56.6″N 112°15′18.9″W) and Kennecott (40°44′52.3″N 112°14′01.2″W), located near the town of Lake Point, Utah, and approximately 6 km apart. With access limited to the Kennecott site in 2015, Lake Point and an additional site, Pine Canyon (40°34′25.9″N 112°14′56.5″W), were sampled during the 2015 season. To obtain larger sample sizes of dead adults late in the season, 30 additional stems were collected at Lake Point in late-June 2014 (resulting in 60 stems collected) and 90 additional stems were collected at Pine Canyon in early-July 2015 (resulting in 120 stems collected), all to be dissected.

Because overwintered stems were often difficult to distinguish between years, random live stems were flagged in the previous summer at each site to serve as examples for stems to be collected. Live stems were haphazardly flagged at each site in the previous spring by selecting stems every 3 meters along transects that radiated from a central point. These flagged stems were among those collected for dissection in the following spring and summer.
Overwintered Stem Dissections

Dissections of collected stems were made in the lab using cutting boards and razor blades to recover overwintered adults. Data recorded included the status of each adult recovered, i.e. alive, dead, or emerged, and the length of each stem dissected. Emerged adults were identified by an empty mine and an associated exit hole. Recovered adults were preserved in ethanol until they could be sexed and measured.

Sexing and Measuring Overwintered Adults

The sex of each live or dead adult recovered from stems was determined under a dissecting microscope by identifying sex-specific rostral and pro-femoral characters described by Carney et al. (2004) and Schat et al. (2007). Measurements of adult body size were made to the nearest hundredth of a millimeter and included the length from directly above the eye to the posterior end of the pronotum, added to the length of the elytra. This combined length excluded the length of the rostrum. To obtain consistent measurements between individuals, all adults were laid on their side during measuring.

Analyses

All analyses were conducted using SAS 9.3. Analyses of variance were used to determine the effects of categorical variables on the density of adults present in stems, adult body size, the percentage of adults that died in stems, and the percentage of those that were female. Analyses of covariance were used to determine the effects of stem density along with categorical variables on weevil mortality and body size. Because males and females occupied the same stems, they could not be considered independent
observations and were separated in several analyses. Paired t-tests were used to compare the frequency of live or dead adults or the body sizes of males and females occupying the same stems. Chi-square analyses were used to determine differences in the number of dead adults with or without exit holes for late-season stem collections. Square-root arcsine transformations were applied to all percentage variables included in analyses.

Results

**Overwintered Stems: Weevil Density, Mortality and Sex Ratios**

Overwintered stems collected in March of 2014 and 2015 varied in weevil density, with between 7.73 and 18.93 adults (both living and dead) per stem occurring on average across sites and years. Very few dead larvae or pupae were found in stems collected in March (less than 4% of weevils had died during development to adulthood in the previous year).

The average density of weevils did not significantly vary between stems collected early, mid, or late in each season for all samples (Table 3.2; 30 stems dissected at each site-year). Thus similar numbers of weevils were estimated to have matured in stems as collected later in the season (at which time many more adults had left the stems, leaving behind exit holes from empty mines within stems; Table 3.2). However, the percentage of dead adults in stems increased throughout season (from 3.6% in March to 25.6% in June 2014 and from 3.9% March to 6.6% to July in 2015) while the percentage that were alive but had not yet emerged from the stem decreased (Fig. 3.1). Across sites and years, 83% of adults had emerged from stems that were collected late in the season. Among
remaining adults, most were dead in stems collected in late June 2014 and all were dead in stems collected in early July 2015 (Fig. 3.1).

In overwintered stems collected in late-March, when few adults had emerged from the stems, the average sex ratio of adults (dead and alive combined) did not differ significantly from 1:1 at all sites-years with the exception of Pine Canyon in 2015, where a 1.33:1 female skewed sex ratio occurred within stems (Table 3.3). Between late March and early May, large numbers of adults began emerging from the stems (Fig. 3.1). More males than females emerged at this time, such that in stems collected in early May, the sex ratio of adults still remaining in the stems was heavily skewed towards females (Fig. 3.2). A two-way ANOVA on the percentage of individuals in a stem that were female revealed a significant effect of month of collection as well as sample (site-year), with a higher percentage of females occurring in stems collected in May versus March (Table 3.4). The nearly significant interaction of month of collection and sample reflects that the degree to which the percentage of individuals that were females increased from March to May varied among site-years (Fig. 3.2).

The number of dead females and males occurring in overwintered stems increased only modestly in 2015 from late March to early July, as did the number of dead males in 2014 from late March to late June (Fig. 3.3). Much higher numbers of females died in the stems at both study sites in 2014 from late March on, such that in late June 2014 (in contrast to early July 2015) there were significantly more dead females than males within stems at both sites (Fig. 3.3 and Table 3.5).
Among those adults found dead in stems in late June (2014) and early July (2015), a large portion of individuals had died after successfully chewing an exit hole (Fig. 3.4). In 2014 (when mortality was especially high), stems from Lake Point had a significantly higher percentage of dead adult females than males associated with an exit hole ($\chi^2 = 13.53$, $N = 162$, $P = 0.0002$), and stems from Kennecott showed a similar, although non-significant tendency ($\chi^2 = 2.4$, $N = 88$, $P = 0.12$). The percentages of dead female versus male adults with an exit hole were similar at both sites in 2015 ($\chi^2 = 0.57$, $N = 34$, $P = 0.45$ for Lake point and $\chi^2 = 0.01$, $N = 95$, $P = 0.92$ for Pine Canyon).

The overall death rate of adults (both sexes combined) in individual overwintered stems was measured as the number of dead adults found in stems in late June 2014, or in early July 2015, versus the total number of adults determined to have inhabited the stems (i.e., as based in part on exit holes for adults that had survived to leave the stem during the spring/early summer). A greater percentage of adults were found dead in a stem as the total number of adults maturing in the stem increased. This pattern was observed at each site in both years (Fig. 3.5), resulting in an overall significant, positive effect of adult density on mortality among the four sites (Table 3.6). However, in individual analyses, this relationship was significant only at Pine Canyon in 2015, for which there was both a large sample size and a large range in the number of adults maturing per stem (Fig. 3.5).

**Body Sizes of Weevil Adults:**
**Variation and Association with Mortality**

Individual adults of *M. janthiniformis* recovered from overwintered stems varied greatly in body size. The average body size (measured as length of live and dead adults)
of males was 4.22 ± 0.025 mm within individual stems collected in late-March while the body size of females was significantly larger, averaging 4.52 ± 0.022 mm in length (paired t-tests resulted in p < 0.0001 comparing average male and female body size with N=30 stems for each of the four site-years; Fig. 3.6). There was little indication that this variation in body size arose from intraspecific competition among individuals within a stem, as the mean body sizes of males and females (both alive and dead) within individual stems collected in late March did not vary significantly with the number of adults inhabiting the stem, although differences in mean body size occurred among the four sets of stems collected at Kennecott in 2014, Pine Canyon in 2015, and Lake Point in both years (Table 3.7).

The potential importance of body size for adult survival and emergence from the stem was assessed by comparing body sizes of adults that were alive in stems in late March (i.e., in overwintered stems collected early in the season before spring emergence) versus those that were found dead in stems collected throughout the season. Body sizes of dead individuals of both male and female adults, as recovered from early, mid and late season stem samples combined, were significantly smaller than body sizes of adults found alive in stems in late March at Lake Point (in both 2014 and 2015) and at Pine Canyon (sampled in 2015) (Fig. 3.7 and Table 3.8). Dead males and females in stems at Kennecott (sampled in 2014) were slightly larger than live adults in stems collected in late March, resulting in a significant interaction of the effects on body size of sample (site-year) with status (alive versus dead) (Table 3.8).
Among those individuals that died within stems, there was no clear, consistent trend in body size occurring for either sex between date of recovery (May versus late June or early July), or between sample (site-year) (Fig. 3.8 and Table 3.9). In addition, there was not a significant difference in mean body size of those that had chewed an exit hole before dying versus those that died before chewing an exit hole. This was true of males in particular. Among females, there was a nearly significant difference in the size of dead individuals that had versus had not chewed exit holes, with those chewing an exit hole being larger on average (Table 3.10).

Discussion

Results of the study by De Clerck-Floate and Miller (2002) indicate that *M. janthiniformis* individuals are intolerant of freezing in the absence of insulating snow cover. At 30% of the sites studied in British Columbia, > 75% mortality occurred as weevils were exposed to temperatures below -28°C. Shat (2008) similarly found high overwintering mortality of *M. janthiniformis* at sites in Montana that was associated with absolute low temperatures below thresholds described by De Clerck-Floate and Miller (2002). In addition to freeze intolerance, weevils exposed to cool summer temperatures as larvae may be especially prone to higher rates of overwintering mortality due to limited development time available to acquire appropriate fat reserves (McClay and Hughes 2007, Arrese and Soulages 2010). As discussed below, this can be investigated by using adult body size as a fitness indicator, because adult size can often reflect larval condition and stored fat reserves (Mirth and Riddiford 2007, Briegel 1990, Carney 2003).
Sites in northern Utah are within the southernmost part of the current range of *M. janthiniformis*, and differ climatically from the sites mentioned above, where weevils have established well but experienced high overwintering mortality. Indeed, sites in Utah have experienced slow *M. janthiniformis* establishment and population growth since original releases were made between 2005 and 2006 at each site (A. Mendenhall, USDA APHIS, personal communication). Currently, there are limited studies conducted on weevil biology in the weevil’s southernmost range in the U.S., including estimates of overwintering mortality. Such studies may help to explain slow weevil establishment in these areas. This study aims to investigate adult mortality in overwintered stems in northern Utah as a potential factor limiting population growth. Furthermore, this study also attempts to describe trends in adult body size to determine the implications of body size on the overall fitness of individuals.

**Adult Mortality**

Overall, overwintering mortality at the study sites in northern Utah, as indicated from early spring stem dissections, is substantially lower than found by De Clerck-Floate and Miller (2002) and Schat (2008) in western Canada and Montana respectively. When averaging among sites-years in the present study, less than 5% of adults were recovered dead in early spring dissections before adults began emerging in large numbers from stems. This indicates that weevils were not exposed to freezing winter temperatures in these years and had adequate insulation by snow cover (De Clerck-Floate and Miller 2002). The absolute low temperature that occurred during the 2013-2014 or 2014-2015 winters in Tooele, Utah was -16.9°C and -18.9°C respectively, and the maximum level of
snow pack was 330 mm in 2014 and 229 mm in 2015. Thus, sites in northern Utah generally had milder winters compared to those in northern regions including British Columbia, potentially leading to reduced overwintering mortality. Also, warm summers occurring at sites in northern Utah likely provided the required minimal time (or degree-days) necessary for larval development to occur (McClay and Hughes 2007). This likely resulted in larger, healthier adults that had acquired the necessary fat reserves to emerge successfully from stems in the following spring (see below) (Arrese and Soulages 2010, Mirth and Riddiford 2007).

During an entire year, overall mortality of adults within stems was low; an average of 16% of adults died and failed to emerge from stems by late in the season. The majority of deaths in stems occurred during spring and summer emergence, and especially so in 2014. Of those that died (including males and females), many of them had successfully chewed an exit hole before dying in both years. This indicates that the timing of emergence from stems may be important for survival, especially for weaker individuals, if fat reserves are indeed limiting after overwintering. Thus, it would be beneficial to exit stems earlier, rather than later in the spring, for weaker individuals. In several observations, adults had even exited half-way from stems before dying and appeared to be stuck in place. In these cases, it could be possible that adults did not chew their exit holes large enough prior to exiting, became stuck in the process, and died in place.

In addition, higher rates of mortality occurred in stems with higher weevil densities, and females died more often before exiting than males, but only in one year
Larval overcrowding may have influenced overwintering mortality by reducing adult body size, and potentially fitness, as observed by Carney (2003). This may also help to explain smaller body sizes among dead individuals compared those living in this study (see below). However, although dead individuals in this study were indeed smaller than those living, there was little evidence that this was a response to larval overcrowding. Females also died in stems at higher rates in 2014 than in 2015. Because females often emerged from stems later in the spring than males to begin feeding and mating, it could be possible that females were especially prone to death before emergence due to higher metabolism of fat reserves in response to higher temperatures (Klepsatel et al. 2016). However, temperatures experienced between the 2014 and 2015 seasons when females began to exit stems were very similar. Other climatic factors and host plant characteristics, such as drought and plant diameter at the development site, could be contributing to the overall variability in mortality observed between 2014 and 2015. These factors were not addressed in this study.

**Body Size Variation**

Male and female M. *janthiniformis* adults collected from northern Utah sites varied similarly in body size to observations made by Toševski et al. (2011) and Carney (2003), in that females were consistently larger than males on all occasions. This is common among many insect species, as females grow at faster rates or for a longer period of time, leading to larger body sizes. In fact, the difference in development rate between the sexes of M. *janthiniformis* likely contributed to the protandry, or early
emergence of males, observed at Utah sites discussed in Chapter 2 (Thornhill and Alcock 1983).

At the study sites in Utah, dead individuals of either sex, as recovered from overwintered stems throughout the season, were significantly smaller than live individuals collected from early March stems for 3 of 4 samples. Because early March stems included both those that would die and those that would emerge, these trends would likely be more pronounced and statistically significant if dead adults were directly compared to live individuals on plants, i.e. those that had successfully emerged from stems. For ectotherms, it is the general consensus that “bigger is better” when referring to adult body size and fitness (Partridge and Farquhar 1983, Kingsolver and Huey 2008, Credland et al. 1986, Juliano 1985). For *M. janthiniformis* adults, smaller individuals, and especially small females, may be especially prone failed emergence from overwintered stems due to limiting fat reserves acquired during larval development (Chapman 1982).

Body size also varied significantly between stem collections made at several sites between two years. These differences in body size between samples are likely the result of many biotic and abiotic factors that may influence the body size of adults (Alcock 1984, Clapham and Karr 2012, Chown and Gaston 2010). Carney (2003) found similar results when comparing the body sizes of males and females between years, and she found that smaller body sizes tended to be associated with stems supporting large numbers of weevils (i.e., high stem densities). Reductions in adult body size may be the result limited resource acquisition during larval development in overcrowded stems (Juliano 1985), but this does not account for body size variation between years or sites.
with similar stem densities. However, while Carney (2003) observed significant reductions in adult body size of either sex occurring in stems with high weevil densities, there was no such indication for this study, although increased stem density appeared to result in increased mortality. Rather than high stem density and intraspecific competition, variation in body size and overall fitness of weevils may be due to variability in host plant quality among sites that warrants further investigation (Awmack and Leather 2002).

Conclusions

Evaluation of adult mortality in stems during overwintering and spring emergence in this study indicates that weevils survive relatively well under Utah conditions. Smaller individuals are especially prone to mortality during spring emergence, but further studies are required to determine the source of body size variation for each sex, including the potential role of host plant quality. Overall, this study suggests that biocontrol of Dalmatian toadflax as it spreads to southern regions should not be limited by high adult mortality during overwintering and spring emergence.

Literature Cited


Tables and Figures

Table 3.1. Sites and dates of overwintered stem collections made in 2014 and 2015.

<table>
<thead>
<tr>
<th></th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lake Point</td>
<td>Kennecott</td>
</tr>
<tr>
<td>Early-season</td>
<td>25 March</td>
<td>25 March</td>
</tr>
<tr>
<td>Mid-season</td>
<td>8 May</td>
<td>8 May</td>
</tr>
<tr>
<td>Late-season</td>
<td>26 June</td>
<td>26 June</td>
</tr>
</tbody>
</table>

Table 3.2. Summary of ANOVA results comparing abundance of weevils in stems collected at early, mid and late intervals during 2014 or 2015 at several sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>DF</th>
<th>F-value</th>
<th>Pr &gt; F</th>
<th>Mean + SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Point</td>
<td>2014</td>
<td>2,87</td>
<td>0.18</td>
<td>0.83</td>
<td>10.4 ± 0.57</td>
</tr>
<tr>
<td>Kennecott</td>
<td>2014</td>
<td>2,87</td>
<td>1.03</td>
<td>0.36</td>
<td>12.3 ± 0.86</td>
</tr>
<tr>
<td>Lake Point</td>
<td>2015</td>
<td>2,87</td>
<td>2.04</td>
<td>0.14</td>
<td>16.4 ± 0.98</td>
</tr>
<tr>
<td>Pine Canyon</td>
<td>2015</td>
<td>2,87</td>
<td>1.07</td>
<td>0.35</td>
<td>7.5 ± 0.67</td>
</tr>
</tbody>
</table>
Table 3.3. Results of four paired t-tests comparing the numbers of males and females (alive or dead) present in overwintered stems collected at several sites in March of 2014 and 2015.

<table>
<thead>
<tr>
<th>Site/Year</th>
<th>df</th>
<th>t Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Point 2014</td>
<td>24</td>
<td>0.27</td>
<td>0.79</td>
</tr>
<tr>
<td>Lake Point 2015</td>
<td>29</td>
<td>0.61</td>
<td>0.55</td>
</tr>
<tr>
<td>Kennecott 2014</td>
<td>19</td>
<td>0.42</td>
<td>0.68</td>
</tr>
<tr>
<td>Pine Canyon 2015</td>
<td>26</td>
<td>2.24</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3.4. Results of a two-way ANOVA of the effect of sample (site-year) and date of collection (March or May) on the percentage of individuals in a stem that were female.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<th>Type III Sum of Squares</th>
<th>F Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>3</td>
<td>0.88</td>
<td>3.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Date</td>
<td>1</td>
<td>6.32</td>
<td>66.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sample*Date</td>
<td>3</td>
<td>0.66</td>
<td>2.32</td>
<td>0.08</td>
</tr>
<tr>
<td>Error</td>
<td>205</td>
<td>19.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>212</td>
<td>27.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.5. Results of four paired t-tests comparing the numbers of dead male and female adults present in overwintered stems collected in June/July of 2014 and 2015 at several sites.

<table>
<thead>
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<th>Site/Year</th>
<th>df</th>
<th>t Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Point 2014</td>
<td>53</td>
<td>-8.46</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lake Point 2015</td>
<td>15</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>Kennecott 2014</td>
<td>21</td>
<td>-5.66</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Pine Canyon 2015</td>
<td>58</td>
<td>-0.19</td>
<td>0.85</td>
</tr>
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Table 3.6. Results from ANCOVA of the effects of sample (site-year) and density on the percentage of adults that were dead by late season stems. A sqrt arcsine transformation was applied to the percentage.

<table>
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<tr>
<th>Source of Variation</th>
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<th>Type III Sum of Squares</th>
<th>F-Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>3</td>
<td>1.34</td>
<td>6.34</td>
<td>0.0004</td>
</tr>
<tr>
<td>Density</td>
<td>1</td>
<td>0.33</td>
<td>0.33</td>
<td>0.03</td>
</tr>
<tr>
<td>Sample*Density</td>
<td>3</td>
<td>0.04</td>
<td>0.01</td>
<td>0.89</td>
</tr>
<tr>
<td>Error</td>
<td>299</td>
<td>21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>306</td>
<td>25.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.7. Results from ANCOVA for each sex of the effect of sample (site-year) and stem density on average adult body size of weevils that were alive or dead occurring within March stems.

### Males

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (site*year)</td>
<td>3</td>
<td>0.51</td>
<td>3.99</td>
<td>0.01</td>
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<tr>
<td>Density</td>
<td>1</td>
<td>0.009</td>
<td>0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>Sample*Density</td>
<td>3</td>
<td>0.09</td>
<td>0.73</td>
<td>0.54</td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>3.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>5.67</td>
<td></td>
<td></td>
</tr>
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</table>

### Females

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<tbody>
<tr>
<td>Sample (site*year)</td>
<td>3</td>
<td>0.48</td>
<td>7.49</td>
<td>&lt; 0.001</td>
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<tr>
<td>Density</td>
<td>1</td>
<td>0.07</td>
<td>3.27</td>
<td>0.07</td>
</tr>
<tr>
<td>Sample*Density</td>
<td>3</td>
<td>0.03</td>
<td>0.54</td>
<td>0.65</td>
</tr>
<tr>
<td>Error</td>
<td>89</td>
<td>1.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>4.31</td>
<td></td>
<td></td>
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</table>
Table 3.8. Results from ANOVA of the effect of status (live or dead) and sample (site-year) on male or female adult body size. Live individuals from March stems are compared against individuals as combined from all early, mid, and late season samples of stems.

### Males

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>3</td>
<td>1.13</td>
<td>6.39</td>
<td>0.0004</td>
</tr>
<tr>
<td>Status</td>
<td>1</td>
<td>2.08</td>
<td>35.12</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sample*Status</td>
<td>3</td>
<td>0.8</td>
<td>4.53</td>
<td>0.005</td>
</tr>
<tr>
<td>Error</td>
<td>141</td>
<td>8.34</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>12.62</td>
<td></td>
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### Females

<table>
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<tr>
<td>Sample</td>
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<td>2.02</td>
<td>9.13</td>
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<tr>
<td>Status</td>
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<td>1.46</td>
<td>19.8</td>
<td>&lt; 0.0001</td>
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<tr>
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<td>3</td>
<td>1.4</td>
<td>6.36</td>
<td>0.0004</td>
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<tr>
<td>Error</td>
<td>173</td>
<td>12.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>16.72</td>
<td></td>
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Table 3.9. Results from ANOVA of the effect of date of collection (mid or late season stem samples) and sample (site-year) on male or female dead adult body size.

### Males

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<th>P-value</th>
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<tr>
<td>Sample</td>
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<td>0.15</td>
<td>0.47</td>
<td>0.70</td>
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<td>Date</td>
<td>1</td>
<td>0.25</td>
<td>2.31</td>
<td>0.14</td>
</tr>
<tr>
<td>Sample*Date</td>
<td>3</td>
<td>0.41</td>
<td>1.27</td>
<td>0.29</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>4.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>5.52</td>
<td></td>
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### Females

<table>
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<tr>
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<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>3</td>
<td>0.55</td>
<td>1.31</td>
<td>0.28</td>
</tr>
<tr>
<td>Date</td>
<td>1</td>
<td>0.0001</td>
<td>0.00</td>
<td>0.98</td>
</tr>
<tr>
<td>Sample*Date</td>
<td>3</td>
<td>0.06</td>
<td>0.13</td>
<td>0.94</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td>10.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>11.22</td>
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Table 3.10. Results of two ANOVAs of the effect of emergence hole presence and sample (site-year) on dead adult body size. Dead adults from mid and late season samples are combined for each sample.

<table>
<thead>
<tr>
<th>Males</th>
<th>Source of Variation</th>
<th>df</th>
<th>Type III Sum of Squares</th>
<th>F Statistic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample</td>
<td>3</td>
<td>0.14</td>
<td>0.32</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Emergence Hole</td>
<td>1</td>
<td>0.12</td>
<td>0.85</td>
<td>0.36</td>
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<td></td>
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<td>0.74</td>
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<td>Error</td>
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<td>10.03</td>
<td></td>
<td></td>
</tr>
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<td></td>
<td>Total</td>
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<td>11.11</td>
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<table>
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<th>Source of Variation</th>
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<td>0.55</td>
<td>0.92</td>
<td>0.43</td>
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<td></td>
<td>Emergence Hole</td>
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<td>0.67</td>
<td>3.34</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Sample*Ehole</td>
<td>3</td>
<td>0.55</td>
<td>0.93</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>182</td>
<td>35.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>189</td>
<td>40.59</td>
<td></td>
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**Fig. 3.1.** Percentage of weevils that were identified as dead, emerged, or alive in stem samples collected on different dates at several sites in 2014 and 2015. N=30 stems for each sample.
Fig. 3.2. Average percentage (±SE) of live individuals that were females within overwintered stems collected in March (dark gray) and May (light gray) at several sites in 2014 and 2015. N refers to the number of live females recovered from 30 dissected stems for each sample.
Fig. 3.3. Comparison of the average number of dead male and female adults recovered from overwintered stems collected at three times of the spring and early summer during 2014 and 2015 at several sites.
Fig. 3.4. Percentage of dead males or females recovered from late season stem collections that had chewed an exit hole before dying. N refers to the total number of dead males or females recovered with an exit hole. To obtain larger sample sizes, additional stems were collected at Lake Point in 2014 (resulting in 60 stems dissected) and at Pine Canyon in 2015 (resulting in 120 stems dissected).
2014

Fig. 3.5. Regression of the number of weevils in a stem (“stem density”) and death rate of weevils (square root arcsine transformation of the percentage of dead individual) for stems collected in late season samples at two sites (Lake Point left circles and Pine Canyon right triangles) in 2014 (unfilled data points) and 2015 (filled data points).
Fig. 3.6. Histogram plotting the frequency of adults in size categories, ranging from 3-5.5 mm, recovered from stems collected in March of 2014 and 2015 at two sites. Each individual measurement is rounded to the nearest quarter of a millimeter. N = 30 stems dissected.
Fig. 3.7. Comparison of adult body size of those identified as dead (collections from early, mid, and late-season stems combined) and alive within stems collected in March, from several sites in 2014 and 2015. Asterisks represent p <0.05 when comparing live and dead individuals in average body size.
**Fig. 3.8.** Comparison of body size of dead adults in stems collected at mid or late-season in 2014 and 2015 at several sites.
CHAPTER 4

DEVELOPMENT OF THE TOADFLAX STEM-MINING WEEVIL, *MECUS JANTHINIFORMIS*, FROM LARVA TO ADULTHOOD: PHENOLOGY MODELS, MORTALITY, AND IMPLICATIONS OF WEED CONTROL

Abstract

One option for the sustainable and long-term control of noxious weeds in rangelands is biological control using herbivorous insects. Post-release monitoring of insect biocontrol agents is crucial for determining program success and future prospects in weed biocontrol. Presented is a study on the development of *Mecinus janthiniformis*, the Dalmatian toadflax stem-mining weevil, from larva to adulthood within stems during two growing seasons at a site in northern Utah where weevils were slow to establish. Living stems were sampled from this site during the growing seasons of 2015 and 2016 and were dissected to determine the developmental and mortality rates of individuals within stems. Results from this study indicate similar phenological trends to previous observations, and in general a high survival rate of weevils to adulthood. However, considerable rates of parasitism were observed, as well as evidence of early exiting by adult weevils from live Dalmatian toadflax stems before overwintering. Overall, the results of this study addressed potential limitations of weevil development in southern regions that experience slow weevil establishment. These findings could be useful in developing new biological control programs of Dalmatian toadflax in the future.
Introduction

Although many efforts have been made to control noxious weeds in rangelands, the most promising method for sustainable weed management in rangelands is currently biological control using herbivorous insects. When successfully implemented, biocontrol programs provide an inexpensive way to control noxious weeds while having little negative impacts on neighboring species and habitat quality (DiTomaso 2000, McFadyen 1998). However, the process of implementing a weed biological control program is time consuming and success is largely unpredictable, as biocontrol agents face unpredictable obstacles after introduction and effective weed control may take years to occur (McClay and Balciunas 2005, McFadyen 1998, Harris 1973, Goeden 1983). Therefore, post-release monitoring of insect and weed populations is crucial in determining effective weed suppression and insect persistence in the environment. In addition, sustained monitoring also provides the opportunity to compare biocontrol programs to identify potential causes of slow and/or ineffective weed biocontrol.

The study presented monitors the development, from larva to adulthood, of a promising biological control agent of Dalmatian toadflax (*Linaria dalmatica* L. (Miller) (Plantaginaceae)), the stem mining weevil *Mecinus janthiniformis* Toševski and Caldera (Coleoptera: Curculionidae), at sites in Utah where weevils were slow to provide effective biocontrol. Dalmatian toadflax is a perennial weed in North America that is highly invasive due to its extensive root system and prolific seed production (Robocker 1974, Sing et al. 2016). As of 2016, nine insect biocontrol agents have been intentionally or unintentionally introduced to control Dalmatian toadflax, but only one, the Dalmatian
toadflax stem mining weevil *Mecinus janthiniformis*, has been successful (Sing et al. 2016, Goulet et al. 2013, Peterson et al. 2005). *Mecinus janthiniformis* has received much attention as a potential biocontrol agent of toadflax due its host specificity and stem mining biology that ultimately lead to widespread biocontrol of Dalmatian toadflax by suppressing growth and reproduction (USDA-APHIS, 1996, Jeanneret and Schroeder 1992).

Internal plant feeders, such as stem miners, are often chosen as biocontrol agents of weeds due to their high degree of host specificity and desired impact on host plants (Ding et al 2014, Connor and Taverner 1997, Van Klinken 2006). Stem mines result from feeding by internal larvae and in some cases, as for *M. janthiniformis*, mines can also serve as dwellings for pupation and overwintering. The use of stem mining insects to combat weeds, especially for perennials, can be very effective by destroying vascular tissue and ultimately preventing nutrient transfer to and from tissues during development (Briese 1996, Peschken and Wilkinson 1981). The evolution of plant mining in insect species is likely the result of intimate interactions between the insect and its host plant, resulting in a high level of host specificity and trade-offs by an insect that has a sedentary lifestyle and protection from exterior elements inside plant hosts (Ding et al. 2014, Connor and Taverner 1997).

The stem mining phenology of *Mecinus janthiniformis* in northern latitudes follows a consistent pattern, with adult weevils emerging from overwintered stems to mate and oviposit eggs into live Dalmatian toadflax stems beginning in May (Toševski et al. 2011, Sing et al. 2016). Upon hatching, larvae of *M. janthiniformis* individuals feed on
internal plant tissues and develop through three larval instars, occurring between mid-May to early-August when individuals begin to pupate inside stems (Sing et al 2016). After pupation, the majority of individuals are adults by late-August and they overwinter within stems until exiting in April. _Mecinus janthiniformis_ individuals are univoltine, (i.e., they have one generation per year); however, there have been observations of exit holes occurring in stems before overwintering, as indicated by chewed exit holes and empty mines (Sharlene Sing, U.S. Forest Service, personal communication). These exit holes have not been fully investigated, and may be associated with early exiting _M. janthiniformis_ adults or parasitoid activity (Jeanneret and Schroeder 1992, Sharlene Sing, U.S. Forest Service, personal communication). Overall, _M. janthiniformis_ phenology is best understood in northern climates where weevils have provided effective weed biocontrol, such as in the Pacific Northwest, but little is known about weevil phenology in southern ranges of their current distribution, including sites in Utah, where weevil establishment has been slow to occur.

The slow and unpredictable establishment of _M. janthiniformis_ on Dalmatian toadflax in southern regions, including the site in Utah studied here, is likely the result of challenging environmental factors that limit weevil survival in these areas (Jamieson et al. 2011, Sing et al. 2008, Park 2013, Goulet et al. 2013, Van Hezewijk et al. 2010, Schat et al. 2011, Carney 2003). Currently, there is little known of these limiting factors that may contribute to slow weevil establishment in their southern range.

This study aims to shed light on weevil survival in arid climates by addressing phenology and mortality of _M. janthiniformis_ individuals as they developed from larva to
adulthood within Dalmatian toadflax stems located in Utah, at a site where weevil establishment was slow to occur. Weevils were monitored intensively in the 2015 growing season at one site to generate a degree-day model that was tested for robustness in a subsequent, less intense study in 2016. As Dalmatian toadflax migrates further south each year, the results of this study could be useful to provide insight for future Dalmatian toadflax biocontrol programs.

**Methods and Materials**

Live Dalmatian toadflax stems were collected during the 2015 and 2016 growing seasons at one site, Pine Canyon (open BLM land: 40°34’25.9’’N 112°14’56.5’’W), and were dissected to determine insect phenology and mortality within life stages. Upon making collections at Pine Canyon in 2015, stems were frozen to hold for later dissection. Stems in 2016 were either frozen or refrigerated, depending on when dissections could be made. If stems could be dissected the subsequent day, stems were refrigerated, but otherwise they were frozen.

**Live Stem Collections**

In 2015, collections were made at approximately one-week intervals beginning on 19 June 2015 and ending on 17 September 2015, resulting in a total of 11 collections. With the exception of two collections in 2015 when only 10 stems were selected (19 June and 10 August), 20 stems were selected and dissected for each remaining sampling date. Stems were sampled in 2015 at Pine Canyon along a 500 m transect that ran parallel (east to west) and 20 m north of four permanent study posts. Stems of 15 cm or more in height
were selected blindly, with no preference for plant characteristics or presence of adult weevils, by collecting the nearest stem approximately every 20 m along the 500 m transect. Because toadflax was abundant along this large transect, the same transect was sampled on each date in 2015.

On 30 August and 17 September in 2015, 10 additional overwintered stems were collected at Pine Canyon. These stems were selected based on the presence of one or more exit hole(s). These targeted stems with exit holes were collected after the random stem sample described above, but along the same transect at Pine Canyon. Stems were selected as described above (stems were examined every 20 m and were at least 15 cm in height), but were collected only if exit holes were present. There was no bias when sampling for stems with certain types of exit holes (see below).

Stem samples were larger in 2016, but with fewer total collections. A major goal in 2016 was to determine the robustness of calendar date/degree-day estimates in 2015 for when during the summer the majority of developing weevils within stems had reached the pupal and/or adult stage (which, along with older larvae, were readily detectable by eye upon stem dissection). Given this goal, sampling did not begin until 7 July 2016, with subsequent samples occurring each week, until 4 August, after which only one remaining sample was taken on 19 September. This resulted in a total of 6 collections. Rather than sampling one transect for each date as done in 2015, 2016 samples were selected on each date along a newly sited transect beginning at the eastern most of the four permanent study posts in Pine Canyon (40°34’25.9”N 112°14’56.5”W). With the exception of two transects, each remaining transect occurred in the northeast quadrant of the site, avoiding...
any permanent study plots, and occurring where Dalmatian toadflax is dense and continuous. The remaining two transects occurred in the southeast and northwest quadrants, also where Dalmatian toadflax presence was continuous and no permanent study plots were intersected. Collections were made every 50-200 m along each 1000 m transect starting 50 m from the study post for each direction. At each collection site along a transect, four stems were selected haphazardly (closest stem in proximity) in each of the four cardinal directions, resulting in an average of 42 stems collected on a sampling date (i.e., from a single transect).

**Live Stem Dissections**

All stems collected in 2015 and 2016 were dissected to recover developing or dead *M. janthiniformis* individuals found within stems. Because the majority of stem collections (all in 2015 and several in 2016) were frozen until dissection, careful determination of live or dead larvae upon initial collection was made by evaluating the condition of the bodies. Dead larvae were generally dehydrated and dark brown in coloration, and all dead pupae or adults were dismembered, underdeveloped (e.g. elytra did not form properly), or covered by a fungus. Because these characteristics were consistently observed in unfrozen stems, they were applied in assessing whether individuals recovered from frozen stems should be counted as alive or dead.

Stems were dissected carefully, using razor blades, by shaving off thin layers of Dalmatian toadflax tissue until recovering an individual weevil. The life stage for each recovered weevil (e.g. larvae, pupae or adult) was recorded as well as its status (alive or dead) upon initial collection. Weevil eggs were not visible in any stem sample so they
were not counted in the study. All exit holes found in dissected stems were recorded, and the best determination was made (as described below) as to whether each exit hole was created by exiting parasitoids or by prematurely exiting *M. janthiniformis* adults. Live and dead parasitoids were also recovered from stems and were recorded for current life stage (e.g. larvae, pupae, or adult).

In 2015, all dissections of randomly collected stems and stems targeted for exit hole presence were made under a dissecting microscope. Given that many weevils were already older instars when field sampling began in 2016 (on July 7), stems were dissected simply by eye (i.e., without a microscope) in 2016. Dissecting stems by eye resulted in fewer observations of small *M. janthiniformis* larvae, parasitoids, dead *M. janthiniformis* individuals, and exit holes. Therefore estimates of mortality, parasitism, and exit hole presence are considered in depth for 2015 only. However, estimates of weevil density for late-season stem samples (i.e. stems collected in August or September when the majority of individuals were live adults or pupae) were analyzed and compared between years because there was likely little bias between recovery rates of live adults or pupae between the two dissection techniques.

**Determination of Exit Holes**

Upon dissecting viable Dalmatian toadflax stems during the 2015 and 2016 seasons, two types of exit holes were observed: those that were circular and approximately 1mm in diameter, and those that were ovate and roughly 3mm in length (Fig. A1). Exit holes were dissected carefully to observe associated contents in the stems. Small circular exit holes were often found with evidence of creation by parasitoid wasps.
(identified as solitary pteromalids or eupelmids; Fig. A2) while exit holes that were larger and ovate were often found with evidence of creation by *M. janthiniformis* adults. However, there were a few exceptions, with exit holes being large and round, or irregularly shaped. These exit holes were most often found containing live *M. janthiniformis* adults. In these relatively few cases, it appeared that these adults were collected during the exiting process and had chewed unfinished exit holes.

Evidence recovered from mines associated with exit holes included the presence of live or dead insects that had not yet emerged from stems but had chewed an exit hole, and physical remains of insects that had successfully emerged from chewed exit holes. These remains included shed exuviae (especially head capsules), weevil carcasses that had been parasitized, and frass indicative of wasp or weevil presence (*M. janthiniformis* usually piles frass on one end of the mine).

Of the mines associated with exit holes found in live Dalmatian toadflax stems, the majority (70-80%) were empty except for the presence of physical remains from wasps or weevils that had exited. However, when exit holes were found with live or dead insects present (i.e., wasps or weevils that had not emerged but likely chewed the exit hole before dying or, if alive, before attempting to emerge) about 90% of these holes were circular when wasps were present, or ovate when weevils were present. In a relatively few cases, small likely incomplete circular exit holes were observed with live or dead *M. janthiniformis* adults. There were no cases in which large ovate exit holes were observed with live or dead wasps.
For this study, both exit hole size and shape and evidence from exit hole remains from stem dissections were used to assign exit hole creation by wasps or *M. janthiniformis* adults when the insects themselves were present. Based on the high degree of association of circular holes with wasps and ovate holes with weevils when insects were present, exit holes could be confidently assigned as created by either insect based on size and shape alone when neither the insects nor their physical remains were present. This confidence was further supported by a >90% accuracy in predicting which insect (weevil or parasitoid) had chewed the exit hole, based on exit hole shape and size alone when stems with exit holes, collected on 30 August and 17 September 2015, were dissected.

**Degree-Day Modeling**

Archival weather data used for degree-day modeling was downloaded from the Utah Climate Center’s database for the Tooele weather station (station ID: USC00428771) between 1 January to 31 December in 2015 and 2016. A single sign model was generated (University of California IMP) using daily minimum and maximum temperatures over a critical temperature of 10°C (developmental threshold used by McClay & Hughes (2007)) and below a horizontal cutoff of 30.8°C (derived from the following equation generated by McClay & Hughes (2007) to describe temperature effects on developmental rate:

\[ y = -0.00006t^2 + 0.0037t - 0.0328 \]
Because it is not clear from McClay and Hughes (2007) whether *Mecinus janthinus* or *Mecinus janthiniformis* was studied to derive this equation, interpretation of results should be made cautiously.

**Analyses**

Statistical analyses were conducted in SAS (SAS 9.3 Institute 2009). A linear regression was used to determine the relationship between Julien calendar date (day of year), and the percentage of individuals found to be larvae. One-way ANOVA, using PROC GLM in SAS, was used for each year to determine the effect of collection date on the mean number of weevils present in stems, to determine any change in weevil density throughout each season. An additional one-way ANOVA was used to determine whether the density of weevils per cm of stems, collected on 30 August and 17 September in 2015 at Pine Canyon, differed between stems with and without exit holes. This analysis pooled data from both dates, and included stems that were targeted based on exit hole presence, and stems collected randomly (without any bias for exit hole presence) from the same transect on the same dates at Pine Canyon. The final statistical analysis was a paired t-test used to compare the average number of weevils recovered from stems between 2015 and 2016 on similar calendar dates.

**Results**

A clear pattern of phenology was observed for developing *M. janthiniformis* individuals within the 2015 growing season when comparing relative proportions of live individuals as larvae, pupae, or adults in stems collected during the season (Fig. 4.1A).
Trends in phenology at the same site in 2016 using calendar dating were similar, but with fewer stem collections (Fig. 4.1B).

During the early summer of 2015, the majority of live individuals in Dalmatian toadflax stems were recovered as larvae (100% on 19 June 2015), and this percentage decreased linearly on subsequent sampling dates (linear regression of larvae percentage by Julien day of year: \( F_{1,9} = 51.79, P < .0001, R^2 = 0.85 \)). It is very likely that these larvae sampled on 19 June 2015 were 1\textsuperscript{st} instars given their small size compared to larvae collected on later dates. By 20 July 2015, the majority of larvae were large, and individuals collected on later dates were no larger, so it is likely that these large individuals observed on 20 July were 3\textsuperscript{rd} instars. There were observations of “medium” sized larvae (i.e. between the sizes of those collected on 19 June and those large individuals on 20 July), found in stems beginning on 6 July 2015, and these were likely 2\textsuperscript{nd} instars. In contrast to 19 June and 20 July when most larvae were likely 1\textsuperscript{st} or 3\textsuperscript{rd} instars, there was no sampling date in 2015 when the majority of larvae recovered from stems were 2\textsuperscript{nd} instars, but these intermediate-sized larvae appeared to be most prevalent in stems collected on 13 July 2015.

Live pupae were first observed in stems beginning on 6 July in 2015 and increased in proportion until reaching a maximum percentage of 39.84 ± 5.61% of live individuals recovered on 20 July. After 20 July in 2015, the presence of live pupae in stems decreased to very low proportions by mid-August until none were observed by September (Fig. 4.1A). The earliest live adults were observed in stems collected on 13 July in 2015, with proportions of adults increasing linearly on each subsequent sampling
date (linear regression of adult percentage by Julien day of year: $F_{1,9} = 52.74$, $P < 0.0001$, $R^2 = 0.85$). On the last sampling date, 17 September 2015, 100% of live individuals had reached adulthood. Overall, a key turning point in *M. janthiniformis* development occurred on 20 July in 2015, when 51% of individuals were pupae or adults. All three life stages (larvae, pupae and adults) overlapped the most on 28 July in 2015 with life stages having close to equal proportions (Fig. 4.1A).

The numbers of dead individuals in stems gradually increased throughout the 2015 season, reaching a maximum average of $39.1 \pm 5.29\%$ of total individuals recovered from 30 August stems (Fig. 4.2A). Total individuals include all that were alive, dead, parasitized, and that had exited stems. In the early half of the 2015 season, the percentage of dead individuals in stems did not vary considerably from 10%. High rates of mortality occurred in the latter half of the season, from 10 August to 17 September, when 18-39% of individuals had died.

Although fewer stem collections occurred in 2016, overall trends in phenology when considering proportions of live individuals within stems were similar to those in 2015 (Fig. 4.1). When considering the percentage of total individuals that were recovered dead in 2015 and 2016, substantially fewer individuals were dead on any corresponding sampling date in 2016 (Fig. 4.2). A mere maximum of $3.77 \pm 1.61\%$ of individuals were dead on 28 July in 2016. Because of small sample sizes, trends in mortality were difficult to observe in 2016 (Fig. 4.2B).
Relative Densities of Live Individuals

Degree-day descriptions of weevil phenology showed clear continuous patterns in weevil development when densities of live larvae, pupae, and adults within stems were compared during the 2015 season (Fig. 4.3A). The overall density of living weevils in stems, including all life-stages, was consistent between sampling dates in 2015, showing no significant variation in the number of individuals recovered in stems among collection dates (Table 4.1 and Fig. 4.3A).

Living larvae were observed on the first sampling date in 2015 and reached a peak density of 12.65 ± 1.69 individuals on the next sampling date, 6 July (Fig. 4.3A; 813 degree-days). The density of live pupae followed a rough bell-shaped curve beginning in early-July and ending in late-August of 2015, with a peak density of 4.95 ± 0.84 individuals occurring on 20 July or at 990 degree-days), when pupae plus adults occurred in nearly equal abundance with larvae within stems. At 899 degree-days, on 13 July in 2015, adults first appeared in stems and increased in presence until reaching a peak density of 8.7 ± 1.19 individuals on 10 August (1262 degree-days). In general, after reaching high densities at 1174 degree-days in early August, the number of adults in stems varied between 6 and 8.7 individuals per stem for the remainder of the 2015 season.

General phenological trends for all life-stages were similar in 2015 and 2016 as described by degree-day modeling, although fewer stem samples were collected in 2016 (Fig. 4.3). However, when comparing the two years, it appears that phenology in 2016 was slightly advanced to observations in 2015, with pupae and adults peaking in density
earlier (i.e., after fewer degree-days had accumulated) in the 2016 season (Fig. 4.3). Pupae and adults were about as abundant as larvae in stems on 14 July 2016, when 855 degree-days had accumulated. Because the first stem sample was not taken until early July in the 2016 season, trends in larval phenology in 2016 are incomplete and difficult to compare to 2015 (Fig. 4.3B); however, it appeared that general trends may be similar although densities of larvae were considerably lower in 2016 stems.

Overall stem density (live and dead combined) varied among dates in 2016 (Table 4.1). However, when removing the unusually high density sample on 28 July in 2016 from the analysis (1081 degree-days), the resulting densities are similar among remaining dates ($F_{4, 198} = 0.91, P = 0.46$). When only comparing late-season stem densities between years (i.e., when the majority of individuals are conspicuous pupae or adults, from late-July to September), density was not significantly different on corresponding sampling dates between 2015 and 2016 ($t_{3} =2.70, P = 0.074$). In contrast, higher densities were observed in early-season stem samples (until late-July) in 2015 than on similar dates in 2016, likely due to differences in the dissection technique employed between the two years (see Methods).

**Densities of Dead Individuals in Stems**

The density of dead individuals in 2015 stems (including all life-stages and those individuals parasitized) increased from the first sampling date to a maximum of $4.05 \pm 0.73$ dead individuals per stem occurring on 30 August or 1552 degree-days (Fig. 4.4). Mortality in 2015 stems nearly doubled on three sampling dates from the previous sampling date, occurring at 813 degree-days (6 July), at 1262 degree-days (10 August),
and at 1552 degree-days (30 August). These points coincide with maximum larval density, maximum adult density, and a small dip in adult density respectively (Fig. 4.3A). Stems collected on all dates during the 2016 season had substantially fewer dead adults present (including those parasitized) than on corresponding dates in 2015 (Fig. 4.4; < 0.5 individuals per stem on all dates in 2016). Although small numbers of dead individuals were observed in 2016 stems, there is a similar trend to 2015 in that the density of dead individuals in stems increased in the latter half of the season.

Because sample sizes of dead individuals were so low in 2016 (Fig. 4.4), mortality trends are only considered for 2015 stem samples. In 2015 stems, 90 to 100% of those recovered dead in the earliest stem samples (between 19 June and 13 July) died as unparasitized larvae (Fig. 4.5). The remaining 10% of deaths occurring between these dates were likely the result of parasitism as indicated by empty small and circular exit holes. Throughout the remainder of the 2015 season, the percentage of dead that died as unparasitized larvae steadily decreased, as more individuals died in later life stages or were parasitized (Fig. 4.5). The percentage of dead pupae fluctuated from 5 to 27% of total deaths (including those parasitized) on all sampling dates from 20 July (when dead pupae were first observed) to the final sampling date on 17 September. Of all life-stages, the fewest deaths occurred during adulthood. A maximum of 20.98 ± 13.51% of dead were adults in stems collected on 10 August, while on all remaining dates this percentage was < 10%.

Comparisons of mortality trends indicate that the greatest number of deaths during the 2015 growing season was likely a result of parasitism (Fig. 4.5; 50% of deaths
in 17 September stems). Of those not considered to be parasitized, the majority of deaths occurred in the larval stage (33% of larvae recovered from all stem samples were dead). During pupation, 17% of all individuals died before reaching adulthood, and only 1.34% of adults died in stems before overwintering.

**Parasitism of *Mecinus janthiniformis***

Parasitism of weevils in 2015 occurred on all sampling dates with the exception of weevils collected on 19 June (Fig. 4.5). Rates of parasitism among weevils that had died generally increased on successive sampling dates throughout the 2015 season, with the exception of stems collected on 3 August, starting from 7.69 ± 7.69 % on 6 July and reaching a maximum of 50.65 ± 8.72% on 17 September (Fig. 4.5). Congruently, the density of parasitoids in stems increased from 0.05 ± 0.05 individuals per stem on 6 July (813 degree-days) to a maximum density of 1.35 ± 0.27 individuals per stem on 30 August (1552 degree-days) (Fig. 4.6).

Likely because sample sizes of recovered parasitoids were generally low on all dates in 2015 (< 1.35 individuals per stem) and several species were recovered, trends in wasp phenology resulted in overlapping life-stages for the majority of sample dates (Fig. 4.7). The earliest indication of wasp inhabitance in stems occurred on 6 July when a single wasp exit hole was recovered (i.e. an exit hole that was small and circular). This indicates that wasps potentially develop and exit stems beginning in early-July. No wasp exit holes were observed in stems collected between 20 July and 10 August in 2015, all wasps were recovered as live or dead larvae, pupae or adults (Fig. 4.7). Wasp exit holes were found again in stems at low rates on 17 August and increased in prevalence on each
subsequent sampling date thereafter until reaching a maximum of 56.55 ± 11.19% of observations of wasps or their exit holes on 17 September.

Live wasp larvae were recovered more consistently in the latter half of the 2015 season, but at low rates, while live pupae were recovered most often in mid-August (reaching 75 ± 25% of all wasps found on 10 August). Overall, live wasp adults were rarely encountered and only occurred in four of eleven collections beginning on 3 August in 2015 (Fig. 4.7).

### Presence of Exit Holes in Live Dalmatian Toadflax Stems

Similarly to above, trends in exit hole presence in live Dalmatian toadflax stems were only evaluated for stems collected during the 2015 season, due to very low sample sizes in 2016. However, even in 2015, instances of exit hole presence in stems were infrequent throughout the entire season (Fig. 4.8A). Starting from 6 July (813 degree-days) when exit holes were first observed, stems had low but consistent exit hole densities until 10 August (1262 degree-days), after which density quadrupled (from an average of 0.1 holes per stem to 0.4 holes per stem) and continued to increase until reaching a peak density of 0.9 ± 0.28 holes per stem on 24 August (1463 degree-days) (Fig. 4.8A). During late August and September in 2015, when exit hole density was relatively high and additional stems were collected that had exit holes (see methods), weevil densities were higher within stems that had exit holes compared to those that did not (one-way ANOVA for stems collected on 30 August and 17 September combined: F\textsubscript{1,58} = 10.72, P = 0.0018).
These observed exit holes in viable Dalmatian toadflax stems were likely created by newly pupated *M. janthiniformis* adults attempting to exit stems, or by exiting parasitoid wasps (Fig 8B). The majority of exit holes observed in Dalmatian toadflax stems up to 17 August of the 2015 season (1369 degree-days) were judged to be created by exited *M. janthiniformis* adults. However, from 24 August (1463 degree-days) to the end of the season, 50% or more of observed exit holes in live Dalmatian toadflax stems were assigned creation by emerged parasitoid wasps (Fig. 4.8B).

**Discussion**

Sites in Pine Canyon have experienced slow weed control since first weevil release in 2006 (*A. Mendenhall, USDA-APHIS, personal communication*); however, weevils had increased to high densities by 2015. At another site not included in this study, at Stockton located 16.4 km southwest from Pine Canyon, weevils were first released in 2009 and density has remained vanishingly low, even after periodic re-releases. Because Dalmatian toadflax is increasingly becoming a problem in southwest regions, including areas in Utah, Colorado, Arizona, and New Mexico (Phillips and Crisp 2001, Zouhar 2003, Ashigh et al. 2010), it is crucial that monitoring studies are conducted to determine the limitations of *M. janthiniformis* in southern territories. This study aims to answer the following questions about weevil activity at Pine Canyon: (1) Is the phenology of larval development to adulthood in southern regions of North America similar to previous observations in northern climates? (2) What are the mortality rates and potential mortality factors of developing weevils in stems before overwintering? (3)
What are the potential causes for premature exit holes occurring in live Dalmatian toadflax stems during a growing season? These questions are discussed below.

**Phenology to Adulthood**

Past observations of *M. janthiniformis* development to adulthood have been made in Europe and at introduced sites in Canada or the American Northwest to describe weevil phenology in these areas (Jeanneret and Schroeder 1992, Toševski et al. 2011, Sing et al. 2016). Observations of weevils in their native range of Yugoslavia demonstrate that adult females begin to oviposit eggs into Dalmatian toadflax stems between late-May to about mid-July (Jeanneret and Schroeder 1992, Toševski et al. 2011). Larvae hatch within one week, and develop through three larval instars, lasting approximately 23 to 34 days, until individuals pupate within stems. Complete development from oviposition to adulthood in Yugoslavia takes approximately 50 to 62 days (Jeanneret and Schroeder 1992). Weevil populations located in the American Northwest have similar seasonal patterns to those found in Yugoslavia (Sing et al. 2016). Larvae develop within stems at these sites from mid-May to early-August, after which the majority of individuals are pupae or adults (Sing et al. 2016). However, a study by McClay and Hughes (2007) found that larvae develop well into October, likely due to cold spring conditions.

Because the study site located in Pine Canyon, Utah generally experiences warmer summer temperatures than those in Canada or in the American Northwest, faster rates of *M. janthiniformis* development during a growing season may be expected to occur at Pine Canyon, leading to observations of adult weevils occurring earlier in the
late summer (McClay and Hughes 2007). When weevil phenology at Pine Canyon was compared to observations in Alberta, development indeed occurred at a faster rate; however, development rates of weevils at Pine Canyon were similar to observations in Europe and in the American Northwest (McClay and Hughes 2007, Jeanneret and Schroeder 1992, Sing et al. 2016, Toševski et al. 2011). Because summer temperatures at Pine Canyon often exceed the 30.8°C upper limit for the maximum development rate of *M. janthiniformis* predicted by McClay & Hughes (2007) (see Methods), similar rates of development may occur at Pine Canyon and these other sites even though temperatures were warmer at Pine Canyon. This however assumes that spring and summer temperatures at sites in Europe and in the northwestern U.S. are generally warm enough to provide similar phenological trends to those at Pine Canyon. This also assumes that temperature has the dominant influence on the weevil’s phenology, rather than precipitation, density dependence, host plant quality or other variables which may indeed also influence phenology, as demonstrated for many insect species (Ellwood et al. 2012, Gibbs and Leston 1970, Jamieson et al. 2012).

To control for possible variations in temperature between sites and years, degree-day models using heat accumulation are often used to predict insect phenology (Herms 2004, Dennis et al. 2014). For all past studies, calendar-dating rather than degree-day models have been used to describe *M. janthiniformis* phenology. During the intensive study at Pine Canyon in 2015, degree-day models were generated to describe *M. janthiniformis* development as a function of degree-days. In addition, simple calendar-dating models were used to compare to previous studies. Overall, both approaches were
consistent with previous observations of *M. janthiniformis* phenology, but previous studies of insect phenology in general suggest that the degree-day models are likely to be more accurate and have better application to future studies.

Although largely consistent, there were several inconsistencies in the degree-day results between years at Pine Canyon. One difference was the forward shift in phenology in 2016 with live pupae and adults peaking in density considerably earlier than in 2015. This could be explained by two factors: first, stems were collected on fewer dates (but with larger sample sizes on each sampling date) in 2016 leading to less accurate estimates of phenology, and second, estimates for degree-days between the years were made only at approximately one-week intervals which may have produced the variability in degree-day accumulations estimated for key events (e.g., first appearance of adults) in the two years.

Another difference between the seasons, not relating to phenology, is the drop in overall density of weevils within stems (including live, dead, and those emerged) between the 2015 and 2016 seasons that was especially prevalent in early season stem samples. As mentioned previously, this was likely due to the stem dissection technique employed between the years.

**Mortality of *Mecinus janthiniformis***

There is little information in the literature on the mortality rates of *M. janthiniformis* individuals during development in Dalmatian toadflax stems, especially in southern regions. In early studies before *M. janthiniformis* was separated taxonomically from *M. janthinus*, mortality of weevils observed in Dalmatian toadflax stems during
development may have been due to unintentionally testing *M. janthinus*, which has low survival rates when reared on Dalmatian toadflax (Toševski et al. 2011). This is especially important to consider when survival in Dalmatian toadflax stems was especially low for developing weevils (McClay and Hughes 2007).

At Pine Canyon in 2015 and 2016, overall mortality of *M. janthiniformis* individuals was low. For both years combined, >65% of individuals in the last stem samples (collected in September) were live adults. Differences in dissection techniques between the years led to much higher mortality rates observed in 2015, but when only comparing the proportions of pupae and adults that survived adulthood before overwintering, mortality rates between the years were similarly low (5.31% of all pupae and adults were recovered were dead in 2015 and only 0.43% in 2016). The difference in the mortality rate for developing pupae and adults between the years was unlikely due to the difference in dissection technique, and more likely accounted for by environmental factors, such as drought and host plant quality, that lead to higher rates of mortality in 2015. Dead larvae were often small and inconspicuous in Dalmatian toadflax stems, so microscope dissections are necessary to recover these individuals to accurately determine mortality rates.

In 2015, mortality rates appeared to nearly double at one point during each of the early, mid, and late season samples. This rapid increase in mortality between subsequent dates may be associated with key events associated weevil development (e.g., larval molting between instars, entering pupation, and emerging as adults) that require energy and may be especially challenging for developing individuals (Cornell and Hawkins
1995, Harcourt 1969). Because sample sizes of dead individuals were low on all occasions, this is merely speculation that requires further investigation.

Of *M. janthiniformis* individuals that died in stems, it is largely unknown as to what mortality factors may be affecting individuals in each life stage. In this study, it was found that mortality was highest for individuals during larval development. Dead larvae were often recovered highly desiccated and brown in coloration. Interestingly, most larvae (and subsequent pupae or adults) were found mining in the top third of stems. Because this is a site of active growth, this may be a preferred feeding site due to softer and more palatable plant tissue present. However, there could also be trade-offs occurring between these preferred feeding sites at the meristems of plants and stem diameter required for successful larval development (Jeanneret and Schroeder 1992). Adult weevils also concentrated their activities on the upper portion of the host plant stem. Roughly 50% of adult weevils were found within the top 10 cm of Dalmatian toadflax stems during stem censusing (see Chapter 2), and this is also were external feeding damage by adults appeared to be most concentrated (*personal observation*).

Within the weevils’ preferred feeding sites, several mortality factors may especially influence larvae as they develop; including overcrowding, plant defenses, disease, and parasitism (Cornell and Hawkins 1995). Larval crowding of *M. janthiniformis* has been found to affect weevil development, leading to smaller adult body sizes and potentially reduced fitness (Carney 2003, see Chapter 3). As for disease and plant defenses, it is often believed that plant mining insects are more prone to mortality by these factors. However, a study by Connor and Taverner (1997) found that
overall, mining insects rarely fall victim to disease or plant defenses compared to external feeding insects. Attack by enemies, including parasitoid wasps, can often lead to high mortality rates of immature insects (Cornell and Hawkins 1995). It could be that parasitoids attack *M. janthiniformis* individuals as larvae and kill the individual before emerging, leading to larval deaths observed in Dalmatian toadflax stems. Further dissections of dead larvae are required to test this hypothesis.

Although pupae and adults also occupy stems, they are likely not vulnerable to the same mortality factors, such as plant defenses, that affect larvae (Cornell and Hawkins 1995). One exception may be larval crowding which can affect adult body size and fitness leading to death before overwintering (Carney 2003). Dead adults, and some pupae, were often found covered in a fungus that was not associated with dead larvae. It is not known if this fungus caused insects to die, or if it arrived postmortem. When considering all weevil deaths recorded in Dalmatian toadflax stems during development, this study indicates that the majority of them are a result of parasitoid attack.

**Parasitoids of *Mecinus janthiniformis***

Past studies evaluating *M. janthiniformis* development within stems mention parasitism by chalcidoid wasps as a mortality factor, but they vary widely in their estimates of the degree of impact on *M. janthiniformis* populations (De Clerck-Floate and Miller 2002, Jeanneret and Schroeder 1992, Toševski et al. 2011, Schat 2008). In Yugoslavia, chalcidoid parasitoids can reduce *M. janthiniformis* presence by 10-80% (Jeanneret and Schroeder 1992, Toševski et al. 2011) while populations in North America have between 0-67% parasitism (De Clerck-Floate and Miller 2002). From these studies,
several families of chalcid wasps have been identified to parasitize *M. janthiniformis* individuals including Eulophidae and Pteromalidae (Jeanneret and Schroeder 1992, Toševski et al. 2011, Schat 2008). In addition, one species of eulophid has been identified by Toševski et al. (2011), *Entedon sparetus* Walker, which is an endoparasitoid of stem-boring weevils (Gumovsky 2007). Other wasps have been identified as ectoparasitoids, but it is the general consensus that many of them attack larvae.

The majority (51%) of dead individuals recovered in late-season stems at Pine Canyon were likely parasitized, as evidenced from recovered wasps and/or wasp exit holes. The steady increase in parasitism observed in 2015 indicates that parasitoids attack *M. janthiniformis* throughout the entire season and not all at once. Also, wasps were recovered from stems in overlapping life-stages. These observations could be explained by a single wasp species having overlapping generations as it attacks *M. janthiniformis*, or more likely by a combination of several wasp species that were present.

Physical observations of parasitoids recovered from stems indicate that several species of wasp may be attacking *M. janthiniformis* at Pine Canyon. Two families have been identified (Pteromalidae and Eupelmidae), but only one individual of the eupelmid wasp was encountered in the stem samples, while the remaining wasps encountered were likely from two species of Pteromalidae (Figure A2). *(personal observation)*. In addition, wasps recovered from stems at Pine Canyon appeared to include both endoparasitoids or ectoparasitoids as evidenced from physical remains of wasps and weevils. In some cases, live wasp larvae were found externally feeding on late-instar weevil larvae or pupae (the pteromalid species that appears to be the most abundant; Figure A2), while in others,
dead weevil pupae or adults were recovered as wasps were exiting their bodies (second pteromalid species; Fig. A2), or were found after wasps had successfully exited (evidenced from weevil carcasses with large open cavities in the body and nearby to a wasp exit hole in stems). Because only one eupelmid wasp was recovered (Fig. A2) with a severely damaged weevil carcass, it is not known if this specimen was an endoparasitoid, or an ectoparasitoid. Overall, when considering all of the evidence, it can safely be assumed that several species of wasps are likely attacking *M. janthiniformis* individuals during development inside Dalmatian toadflax stems at Pine Canyon.

**Premature Exit Holes**

Currently, there is no documented evidence describing premature exit holes occurring in live Dalmatian toadflax stems before spring emergence by *M. janthiniformis* adults. However, there have been anecdotal observations of exit holes occurring in live stems during a growing season in the U.S., but they have not been fully investigated (*Sharlene Sing, U.S. Forest Service, personal communication*).

Exit holes that are present in Dalmatian toadflax stems at Pine Canyon were likely created by exiting parasitoid wasps or by newly pupated *M. janthiniformis* adults attempting to exit stems before overwintering (Fig. A1). If temperatures were sufficiently warm, it is possible that adults were able to accumulate the degree-days necessary to begin a second generation, and therefore exited from stems to feed and find mates.

However, while exit holes were observed in 2015 stems, they were mostly absent in 2016 stems at the same site. Because degree-day models for the two years resulted in similar trends in seasonal accumulation of degree-days and weevil phenology, the lack of exit
holes in 2016 stems is peculiar. This lack of exit holes may hence be due to the dissection technique or other factors, such as the density of weevils within individual stems, which may influence weevils to exit, rather than due to temperature differences between the seasons. Indeed, when weevil densities were compared between stems with and without exit holes present in late-season 2015 samples, it appeared that weevil density was significantly higher within stems that had exit holes. Thus, it could be possible that weevils in 2015 were avoiding intraspecific pressure, and potential overwintering mortality (see Chapter 3), by escaping high density stems before overwintering. However, it is important to note that no live adults were found on Dalmatian toadflax stems and foliage during the fall of either year.

If weevils were beginning a second generation during the fall at Pine Canyon, it’s possible that this could be occurring at other sites in North America. At any location, early exiting adults would be exposed to cool fall temperatures and would likely perish before producing viable offspring. This may have important consequences on weed biocontrol if this trend becomes more prevalent in the future. On the bright side, weevils may be exiting from inhospitable stems to overwinter in the soil, and could potentially survive until the following spring. Overall, more field and laboratory studies are necessary to explore these possibilities and to confirm early adult exiting at other sites.

Conclusions

Interesting findings of this study include similar trends in weevil phenology to previous studies, low mortality rates of developing individuals, parasitism, and early exit
hole presence in live Dalmatian toadflax stems. Overall weevil phenology between years at Pine Canyon was consistent and similar to past studies, and overall mortality of developing weevils was low. This leads to the conclusion that the phenology and survivorship of *M. janthiniformis* bode well for the successful establishment of weevil populations and effective weed control at the study site in Utah. However, this study found low rates of parasitism and early adult exiting, and these activities could pose serious threats to biocontrol efficacy in the future and thus warrant further investigation. Overall, the degree-day models of phenology and estimates of weevil mortality produced by this study could be utilized in predicting key events in weevil biology and weevil performance, and hence are useful results for practitioners of weed control in developing new biocontrol programs for Dalmatian toadflax as it spreads to warmer territories.

**Literature Cited**

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Jeanneret, P., and D. Schroeder. 1992. Biology and host specificity of Mecinus janthinus Germar (Col.: Curculionidae), a candidate for the biological control of yellow and


Tables and Figures

**Table 4.1.** Results of a one-way ANOVA analysis determining the effect of sampling date on stem density of developing weevils during the 2015 and 2016 seasons.

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Fig. 4.1. Relative proportions of developing larvae, pupae and adults of those recovered alive in stems collected during the 2015 (A) and 2016 (B) seasons at Pine Canyon.
Fig. 4.2. The percentage of individuals found dead of the total number of individuals recovered in stem samples collected during the 2015 (A) and 2016 (B) seasons at Pine Canyon. Dead includes all dead larvae, pupae, adults and those parasitized.
**Fig. 4.3.** The average relative densities of living *M. janthiniformis* individuals within the larval, pupal, and adult life-stages per stem collected over the 2015 (top) and 2016 (bottom) seasons at Pine Canyon. Stems in 2015 were dissected under a microscope while those in 2016 were dissected by eye.
Fig. 4.4. Average density of dead weevils (life stages combined) by degree-day accumulation in stems collected during the 2015 (solid line) and 2016 (dotted line) seasons.
Fig. 4.5. Proportions of dead weevils that died as larvae, pupae, adults, or were presumed parasitized in stems collected during the 2015 season.
Fig. 4.6. Average number of parasitized weevils by degree-day accumulation in stems collected during the 2015 season.
Fig. 4.7. Life-stage comparison of parasitoid wasps recovered in 2015 stems including those that died and those that exited stems.
Fig. 4.8. Average density of total exit holes observed on stems collected during 2015 at Pine Canyon by degree-day accumulation (A) and of that the average number of exit holes chewed by *Mecinus janthiniformis* and parasitoid wasps (B).
CHAPTER 5

CONCLUSIONS

The factors limiting effective biological control of Dalmatian toadflax using *Mecinus janthiniformis* are largely unknown in the weevil’s current southern range, including sites in Utah, where biocontrol has been slow to occur. There are likely many interactive abiotic and biotic factors that hinder the ability of *M. janthiniformis* to attack Dalmatian toadflax in these regions, including sites in Utah, but two impeding factors may have been improper phenology and increased rates of mortality during a life cycle. The warm and dry climate of sites in Utah, compared to those in northern regions, could have potentially influenced the phenology of *M. janthiniformis* individuals by increasing rates of development leading to asynchrony with the biology of Dalmatian toadflax while also increasing the mortality of weevils due to exposure to high temperatures and desiccation. Using data collected from field surveys and stem dissections, this study aimed to investigate the phenology and mortality of weevils at southern sites in Utah to help explain slow weed control in the region, and to infer on future biocontrol programs of Dalmatian toadflax. The major conclusions and applications of this study, as pertaining to my initial hypotheses, are discussed below.

Overall I predicted that the phenology and mortality of *M. janthiniformis* individuals during a lifecycle would be well suited to provide effective control of Dalmatian toadflax at sites in northern Utah where the weevil was slow to establish. My initial hypotheses were: 1) the phenology of *M. janthiniformis* at the study sites in Utah would be well synchronized with Dalmatian toadflax although weevil phenology may
differ from past observations made at other sites, 2) degree-day models of weevil phenology would provide more consistent results between sites and between years in this study compared to models based on calendar dates, and 3) compared to previous studies, the mortality of *M. janthiniformis* individuals would be higher during summer development to adulthood but lower for overwintering adults due to hot and dry summers and milder winters respectively.

As described in Chapter 2, models of *M. janthiniformis* phenology as adults on the host plant showed consistent results between sites and between years in Utah, but were especially consistent when modeled using degree-days. One interesting discovery was the difference in phenology between the sexes, with males emerging from overwintering sites and peaking in density on the host plant before females. This apparent protandry has not been described for *M. janthiniformis*, but is likely occurring at other sites spanning North America where the weevil is present. Although the protandry described for *M. janthiniformis* is an interesting phenomenon, it could potentially hinder effective biocontrol if collections of weevils for redistribution are made early in the season when the majority of individuals are male. Overall phenological patterns of *M. janthiniformis* were fairly consistent to past studies, even including those made in British Columbia and in Europe, with one exception being a slightly earlier arrival of adults from overwintering sites on the host plant in Utah. Although weevil phenology in Utah was slightly different than past observations made in northern regions, weevils tended to be well synchronized with the biology of Dalmatian toadflax in Utah, possibly due to a preference for tall, mature stems.
Mortality of adults during overwintering and spring emergence was addressed in Chapter 3, and as predicted, mortality was relatively low during the winter compared to past studies made in northern regions. About 4% of weevil adults died in stems that were collected in early-March, but this percentage increased throughout the spring and early summer as adults exited stems. By June or July the mortality of adults in overwintered stems increased to 7-25%. Of these adults that survived winter but failed to emerge from stems, many were found in stems with high weevil densities, appeared to be smaller in size than those successfully emerging from stems, and had chewed an exit hole before dying. However, there was little evidence of an effect of intraspecific competition on the body size of adults developing in high density stems (as found in past studies), so there may be other factors not investigated in this study, such as host plant quality and allocation of fat reserves, which may have influenced body size and consequently mortality of adults as they overwintered and exited stems in the following spring.

In Chapter 4, the phenology and mortality of weevils, from larvae to adulthood, was addressed for individuals developing within live Dalmatian toadflax stems at one site in Utah. Unlike my predictions, weevils in Utah had high survival rates and phenological patterns that were similar to observations made in northern regions during summer development. At the study site in Utah, the majority of larvae pupated by late-July and reached adulthood by early-August, and at least 65% of individuals found in late-season stem samples were live adults before overwintering while the remaining 35% were dead, largely as a result of parasitism by solitary chalcidoid wasps (51% of those dead were parasitized). Parasitism has been mentioned in past studies investigating *M.*
*Janthinajanthiniformis* mortality, but the impact of parasitism on weevil population varies widely. In addition, this chapter identified and quantified the presence of exit holes occurring in live Dalmatian toadflax stems during the summer. These exit holes were likely created by exiting parasitoid wasps, or by adult weevils exiting stems before overwintering. Weevils may have exited early from stems of especially high weevil densities in an attempt to avoid intraspecific competition, and potentially overwintering mortality, which was observed for overwintered adults within stems occupied by a large number of weevils. Although exit holes were observed at low rates (maximum of 0.4 exit holes per stem), activities by parasitoids or early exiting adults could pose serious threats to biocontrol efficacy in the future and thus warrant further investigation and monitoring.

Overall, this study supported my hypotheses in concluding that the phenology and mortality of *M. janthiniformis* during its lifecycle appeared to be well suited for effective biological control of Dalmatian toadflax in Utah. Weevils in this study were well synchronized in their phenology with that of its host plant and overall mortality was low during development (>50% survival from larval development to spring emergence by adults). Thus effective Dalmatian toadflax suppression in Utah, and in other southern regions experiencing similar climates, will likely not be limited by poorly synchronized phenology and increased mortality of *M. janthiniformis* during overwintering and summer development. However, there may be other limiting factors not investigated in this study, such as drought and host plant quality, which may have impacted the weevils’ ability to provide effective biocontrol of Dalmatian toadflax in Utah and in other southern regions experiencing slow weed suppression. Future research investigating these and
other limiting factors would be helpful in explaining the inconsistencies of Dalmatian toadflax biocontrol using *M. janthiniformis* in North America.

The primary applications of this study include estimates of mortality of weevils in southern regions, and the development of degree-day based models that can better predict weevil phenology. Models of phenology and mortality estimates of weevils may be especially useful to practitioners of biological control in predicting the performance and key biological events of *M. janthiniformis* in new territories. These applications may hopefully improve the confidence and efficacy of implementing Dalmatian toadflax biocontrol programs in the future.
Appendix. Examples of Exit Holes Found in Live Dalmatian Toadflax Stems, and Photos of Parasitoids of *M. janthiniformis*

**Fig. A1.** Examples of exit holes chewed by adult parasitoid wasps (1) and by *Mecinus janthiniformis* adults (2).
Fig. A2. Photos of *M. janthiniformis* parasitoids including (1) a Pteromalidae endoparasitoid adult, (2) a Pteromalidae ectoparasitoid larva, and (3) an adult Eupelmidae wasp.