## Faculty of Science

## WHEN YOUR DINNER BITES BACK: ALSIKE CLOVER AS A HOST FOR COLEOPHORA DEAURATELLA

2015 | AMANDA JORGENSEN

**B.Sc. Honours thesis** 



THOMPSON RIVERS

#### WHEN YOUR DINNER BITES BACK: ALSIKE CLOVER AS A HOST FOR COLEOPHORA DEAURATELLA.

by

#### AMANDA JORGENSEN

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This thesis has been accepted as conforming to the required standards by:

Lyn Baldwin (Ph.D.), Thesis Supervisor, Dept. Biological Sciences

Jennifer Otani (M.Sc.), Co-supervisor, Agriculture Canada

Eric Littley (Ph.D.), Examining Committee member, Dept. Biological Sciences

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#### ABSTRACT

Thesis Supervisors: Dr. Lyn Baldwin, Jennifer Otani (M. Sc.)

Understanding host relationships between invasive species and their non-native hosts has important economic and ecological considerations. The red clover casebearer moth (RCCB), Coleophora deauratella Lieneg and Zeller (Lepidoptera: Coleophoridae), is an invasive red clover seed pest introduced to Canada in the 1960's. RCCB larvae feed on developing seed from June to late August then overwinter in crop debris. RCCB can cause seed yield losses of  $\geq$ 80% in second-year seed stands of red clover. There are no registered pesticides for RCCB. Both larvae and adults have been reported in alsike clover fields but little damage is seen on these plants, and female moths display lower fecundity on alsike clover. The purpose of this research was to compare the larval abundance and development of RCCB on red clover (Trifolium pratense) and alsike clover (Trifolium hybridium). I also wanted to compare moth species diversity and larval abundance in red and alsike clover seed crop fields and volunteer clover patches. In addition, host quality was assessed by rearing field-collected larvae on laboratory-grown clover flower heads and observing growth and development. No larvae were recovered from alsike clover fields or volunteer alsike patches, while 338 larvae were recovered from red clover fields and volunteer patches. Larvae reared on alsike clover had a 30% mortality rate during the rearing period, while larvae reared on red clover had a 5% mortality rate. More weight was gained by larvae reared on red clover (mean weight gain = 0.00438 grams, n=19) compared to larvae reared on alsike clover (mean weight gain= 0.00274 grams, n=109, t = -2.9093, p= 0.01407). This suggests that the difference in damage and fecundity may be due to lower host quality.

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#### **1. INTRODUCTION**

The United States Invasive Species Advisory Committee defines invasive species as "a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health" ([NISC] The National Invasive Species Council 2006). While in many cases introduced species fail to establish, the impacts of species successfully establishing varies. Some introduced species do not become invasive, but others displace natives and/or cause major economic harm (Williamson and Fitter 1996; Strauss et al. 2006). Of the proposed methods by which a species invades, the enemy release hypothesis and increased competitive ability hypothesis have the most support. The enemy release hypothesis states that species populations are controlled by co-evolved natural enemies like herbivores, predators and parasites. When the species enters a new geographic range, the population can increase in the absence of control (Colautti et al. 2004; Liu and Stiling 2006). The increased competitive ability hypothesis, in comparison suggests that invasive species are better competitors than natives due to phenotypic plasticity or traits that are better able to take advantage of the new environment (Colautti et al. 2004; Davidson et al. 2011).

Invasive species encounter and create new ecological relationships in their new range, including competitive and multi-trophic relationships. Overall, herbivore fitness is tightly linked to host plant quality (Awmack and Leather 2002). Measures of host plant effect on fitness that have been frequently cited in the literature include: larval and adult growth, potential and realized fecundity, survival, and development (Awmack and Leather 2002). Plant host quality affects fitness through two major mechanisms: toxicity of secondary

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metabolites or decreased digestibility (Kessler and Baldwin 2002; Knolhoff and Heckel 2014). The effectiveness of these two mechanisms depends on whether the herbivore is a specialist or a generalist. Monophagous species that specialize within a single genus typically are more adapted to host secondary compounds. This is less true for oligophagous species that specialize across several genera and even less true for polyphagous species that feed on hosts from several different families (Bernays and Graham 1988; Howe and Jander 2008; Gripenberg et al. 2010).

Host quality has been shown to influence larval development time of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae): development varied from 26.60 days on corn to 35.07 days on tomato (Liu et al. 2004). On different varieties of soybean *H. armigera* had a range in fecundity between 177.10 eggs/individual on the variety 356 and 582.7 eggs/individual on the variety DPX (Naseri et al. 2009). *Spododoptera exigua* Hübner (Lepidoptera: Noctuidae) is another polyphagous insect that has different feeding and development times on lower quality hosts. On cauliflower 78% of *S. exigua* larvae survived to adulthood compared to 49 and 32% on peas and wheat. These larvae also differed in development time from egg to adult on the three plants: 24 days on cauliflower, 28 days on peas and 33 days on wheat (Saeed et al. 2010).

The red clover casebearer moth, *Coleophora deauratella* Lienig and Zeller (Lepidoptera: Coleophoridae) is a widespread invasive pest. The moth is native to Asia Minor, Turkestan and Europe (Hammer 1937), and through an unknown vector was introduced to North America in 1962 and to Canada in 1970 (Landry 1991). *C. deauratella* was first reported in the Peace River region in 2005 (Yoder and Otani 2010).

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The main host of *C. deauratella* is red clover (*Trifolium pratense* Lam) (Ellis and Bjørnson 1996). Larvae also have been observed on alsike clover (*Trifolium hybridium* L.), stone clover (*Trifolium arvense* L.) zigzag clover (*T. medium* L.) and white clover (*T. repens* L.) (Hammer 1937). *C. deauratella* has also been reported to use knapweed (*Centauria* spp. L.) as a host, but this has never been tested or confirmed (Hammer 1937).

Thus the host range of *C. deauratella* includes plant species introduced to North America and those restricted to Europe. Encountering a new environment creates different challenges and opportunities which potentially restrict, enlarge or shift host use. Host range shifts can occur due to introduction of a high nutrition host (Carroll et al. 1997; Strauss et al. 2006), to take advantage of unexploited niches (Thomas et al. 1987), close host relatedness (Turner et al. 1987) or during high intra or inter-specific competition (Dennill et al. 1993).

Seed-mining coleophorids, like *C. deauratella*, display a high degree of host fidelity, possibly due to high interspecific competition (Bucheli et al. 2002). In North America *C. deauratella* specializes on a naturalized host, which may have changed the ecology of the interaction. In North America *C. deauratella* has escaped some competitors common in Europe, such as *Apion trifolii* and *A. apricans* Herbst (Coleoptera: Apionidae ) (Markkula et al. 1964; Nyabuga et al. 2015). But many other clover seed-feeding insects have also been introduced to North America including: *Hypera nigrirostris* and *H. meles* F. (Coleoptera: Curculionidae), *Bruchophagus roddi* G. (Hymenoptera: Eurytomidae), *Coleophora mayrella* Hübner and *Coleophora trifolii* Curtis (Carrillo and Dickason 1963; Landry and Wright 1993; Weiss and Gillot 1993). The potential effect of these interactions on the host range of *C. deauratella* has not been investigated. Forage crops, including legumes and grasses, are grown typically for animal feed. There is a large market for these crops, making the demand for forage seed high. The Peace River region is one of the few major forage seed producing regions in the world (Wong 2010). The region exports seed to be grown for animal feed primarily to the United States, and in the last ten years has averaged 4.48 million pounds of red clover seed exported per year (Wong 2012). Legume crops grown for seed in the Peace River region are red clover, alsike clover, sweet clover and alfalfa (Wong 2010) . Red clover is an important crop that has long been grown for pasture, hay, silage or tilled back into the earth to provide nitrogen as a green manure (Fairey 1985). Prices for alsike and red clover have both increased since 2014 (Wong 2015a), but even with historically high prices, acres of clover grown in the Peace region are low (1,285 acres in AB in 2014), and decreasing (-41.3% from 2013 to 2014) (Wong 2015b). Although this is partly due to high commodity prices, and damage from *C. deauratella* is also a factor (J. Otani, personal communication).

*C. deauratella* can be highly damaging to seed yields. Fourth instar larvae can consume 1-2 seeds per day and the species can cause seed yield losses of 80% or more (Ellis and Bjørnson 1996; Evenden et al. 2010). Ellis and Bjørnson (1996) suggested that a severe infestation can be classified as one larva per four heads surveyed on a field. Average numbers of larva per head examined in Ontario in 1989 were 1.0-2.3, and in 1990 were 1.8-2.4 (Ellis and Bjørnson 1996). *C. deauratella* is more abundant in second year clover stands than first, and in Finland is more abundant in wild *T. pratense* than cultivated *T. pratense* (Markkula et al. 1964).

There are no registered pesticides for *C. deauratella* (Mori and Evenden 2014a). Small plot trials were conducted in 2007 with Decis<sup>™</sup>, and while a small increase in yield was shown it was still below industry standard with low seed quality and germination (Yoder and Otani 2010). Pesticide applications during clover flowering can also cause a greater yield loss than insect pests because of decreased insect pollination (French 1972). Pheromone trapping has been successfully used to monitor phenology and seasonal peaks (Mori et al. 2014) and pheromone mating disruption has been examined as a control method (Mori and Evenden 2014a; Mori and Evenden 2014b). Currently there is no management strategy for *C. deauratella*.

Understanding the relationship between preference and performance in a pest species provides knowledge that can become part of a management strategy. Most insects have ovipositional preferences between species, cultivars or crop stages that can be ranked in order of desirability (Thompson 1988). Highly ranked plants can be used as trap crops that protect target crops through one of two main mechanisms. The trap crop either prevents the pest from reaching the target crop at all, or concentrates the pest in a small area where insecticidal or other action can be concentrated (Hokkanen 1991). There are examples in the literature of plant species that are highly desirable to the target pest but do not allow for larval development and are potential dead-end trap crops (Shelton and Nault 2004; Khan et al. 2007; Karungi et al. 2010; Badenes-Perez et al. 2014). The brassicaceous plant Barbarea vulgaris R Br. is not only more attractive to *Plutella xylostella* than *Brassica oleracea* L. but also prevents development and survival of larvae due to high saponin content (Badenes-Perez et al. 2014). Saponins are a class of secondary metabolites that are also present in alsike clover (Pérez et al. 2013). Both C. deauratella larvae and adults have been observed in alsike clover fields, yet little damage is seen on these plants and female moths display lower

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fecundity on alsike clover (J. Otani, unpublished data). This study begins examining the potential for use of alsike as a dead-end trap crop by studying abundance larval development.

*Coleophora deauratella* has been misidentified many times in the literature, including a preliminary description of biology in Hammer (1937) where it is misidentified as *Coleophora spissicornis* Haw (syn. *Coleophora mayrella* ) (Landry 1991). This is likely due to the fact that coleophorid moths have large lamellate scales that rub off easily, removing key identification features. Correct identification of damaged specimens can only be accomplished by either examination and dissection of adult genitals (Landry and Wright 1993) or through genetic analysis. There are three coleophorid moths in the Peace River region that are nearly identical: *Coleophora deauratella, Coleophora mayrella* and *Coleophora trifolii*. All three species feed on Fabaceae but each appears to specialize on different host species (Landry and Wright 1993). *Coleophora mayrella* specializes solely on white clover, *Trifolium repens* (Landry and Wright 1993), so correct species identification in this study was critical.

The primary objective of this study was to examine the host preferences and performance of *C.deauratella* on *T. pratense* and *T. hybridium*. Larval abundance in clover seed fields and volunteer clover patches were compared. While fourth instar larvae can move between flowerheads, early instar larvae are quite small and less mobile (J. Otani, personal communication). Larval abundance was measured a few weeks after the flight period began. Host quality was determined by rearing field collected larvae on laboratory-grown clover flower heads and observing growth and development.

#### **1.1 OBJECTIVES**

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- 1. To determine if there is a difference in abundance and density of *C. deauratella* in seed crop fields of alsike and red clover and the volunteer alsike and red clover in adjacent ditches.
- 2. To determine if there is a host quality difference between alsike and red clover through comparing larval development and growth of *C. deauratella* on alsike and red clover.
- 3. To determine the proportion of *C. deauratella*, *C. mayrella* and *C. trifolii* occurring in alsike and red clover growing in the Peace River region of Alberta.

#### 2. MATERIALS AND METHODS

#### **2.1 STUDY SPECIES**

*Coleophora* is a genus of seed and leaf-mining, micro-lepidopterans. The genus is placed in the family Coleophoridae, which belongs to the superfamily Gelechiidae (Landry and Wright 1993; Bucheli et al. 2002). Members of the genus feed internally on their host plant as early instar larva, then build a case constructed of silk and plant materials. The case is carried for the duration of the larval instars and pupation occurs inside the case (Bucheli et al. 2002).

Female *Coleophora deauratella* moths lay eggs from late June until late July on the exterior of the calyx of the clover floret. The eggs hatch and the larvae either burrow into the floret or enter through the tip where they will feed on the developing ovule (Hammer 1937; Landry 1991; Ellis and Bjørnson 1996). The larva will continue to feed until late August or September. This pest is univoltine, and overwinters as a fifth instar larva inside the case it constructs (Hammer 1937; Ellis and Bjørnson 1996).



Figure 1 A. *Coleophora deauratella* adult male (Photo source: Landry 1991). B. *Coleophora deauratella* larval case and larva

#### 2.2 STUDY AREA

The Peace River region extends from 55 ° to 58 ° North and 116° to 120° West and spans the BC-AB border. Originally consisting of aspen parkland, the landscape is now dominated by agriculture. Most agricultural land acreage is dedicated to the production of commodities such as canola (*Brassica napus*), wheat (*Aestivum triticum*), barley (*Hordegum vulgare*) and peas (*Pisum sativum*) (Alberta Agriculture and Forestry 2015). The Peace River region is also one of the largest forage seed production regions in the world, second (by area) only to the Willamette valley in Oregon (Wong 2010). In 2006 the Peace River region (found in both BC and AB) represented 247,788 acres of legume and grass grown for seed (Wong 2010).

This study was conducted in the Falher area in the Alberta Peace River region, which receives 400-450 mm of rain annually and has an annual growing season that averages 120-135 days annually(Wright 2003; [AAFC] Agriculture and Agri-foods Canada 2010). The

July daily mean temperature averages 15 to 16°C while the January daily mean temperature averages – 18 to-16°C (Wright 2003).

Within the study area, the four collection sites were all cultivated clover seed crop fields located in the Falher area, near the towns of Girouxville (2014-46 Lavadiere, 2014-47 Limoge, 2014-48 Rochon) and Guy (2014-49 Chaibos). Three red clover fields (cultivars common and altaswede) and one alsike clover field (cultivar aurora) were sampled. All fields were seeded in 2013. All sites were one quarter section in size (approx. 64 hectares) except for 2014-47 Limoge, which is located on the home quarter, and therefore smaller. All sites were less than 10 km apart, except for 2014-49 Chaibos, which was 34 km away from the next nearest site. Ownership of each site was different. Slope and aspect for the sites were not gathered, as the Falher region of Alberta is extremely flat. Pesticide application information for sites was not available.



Figure 2. Map of study location (Photo source: Labour.gc.ca Alberta Zone map). Area circled encompasses all collection sites.

#### 2.3 FIELD SAMPLING

#### 2.3.2 LARVAL FIELD ABUNDANCE

Flower heads were collected from four sites on 23-July-2014. Flowers were chosen as a surveying method for larval abundance as in Markkula *et al.* (1964) and Mori *et al.* (2014). At each site, flower heads were collected from two sources. The first source was collected from cultivated clover plants in the seed crop field. Flower buds were hand-picked from the field in a 180 degree "U" shaped pattern (Figure 3, thick arrow). Ten paces were taken into the field from the field edge, and then one bud per pace was collected for ten paces. Flower buds were alternately taken from the top of the plant and lower along the stem. After taking ten paces collecting flowers, I walked another ten paces to increase dispersion in the field. This pattern was repeated a total of five times. Overall, flowers from each field were collected over a distance of fifty paces, approximately 50m.

At each site, the second source of flowers was clover of the species alternate to the seed crop species, growing in the ditch adjacent to the crop field edge (Figure 3, narrow arrow). As volunteer clover was spread throughout the ditch in distant patches of one or two plants fifty buds were taken from the closest volunteer clover plants in a straight line parallel to the field edge. The distance paced out to collect 50 flower buds varied from 5 m to 15 m.



# Figure 3. Diagram of flower head collection pattern. The blue arrow represents the collection pattern of the main seed crop flowers. The red arrow represents the collection pattern for the volunteer clover.

Flower buds were placed into labelled paper bags as they were collected. The bags were then stapled shut and transported back to the laboratory in a cooler. The bags were left overnight in a walk-in cooler with an ambient temperature of 1°C. The next day flower heads were separated individually into plastic 1 oz cups lined with black cardstock (Figure 4). The cups were attached to clear perspex sheets with double sided tape which were placed vertically in a container. This allowed all containers to receive ambient light. One drop of reverse osmosis water was added to each container every second day to prevent desiccation.



Figure 4. Red clover buds collected from fields being individually monitored for larval activity in plastic cups.

Larval activity on the flower buds was monitored daily for sixteen days, and then every second day for fourteen days as larval emergence decreased. Any larvae that were visible moving on the flower bud or crawling loose in the plastic cup were removed from the cup and weighed individually with a Mettler Toledo analytical scale. For each bud the number of larvae emerged per flower bud each day was recorded.

#### 2.3.1 ADULT FIELD MONITORING

Adult moth populations at study sites were monitored using commercially available pheromone-baited unitraps (Figure 5). Green unitraps baited with a synthetic blend of Z-7-dodecenyl acetate and Z-5-dodecenyl acetate, the female-released *Coleophora deauratella* pheromone(Evenden et al. 2010), were placed at the field edge of all clover sites. Two traps were placed at each site spread 50 metres apart. Traps were emptied weekly and moth numbers were counted by AAFC students as part of AAFC's red clover casebearer study. Subsamples of 20 moths were taken from each trap each week. The 20 least damaged

specimens were selected for dissection. Each specimen in the subsample was partially dissected to fully reveal genital traits for descriptions in Landry (1991).



Figure 5. Pheromone-baited green unitrap in a red clover field.

#### **2.5 HOST PLANT MAINTENANCE**

Red and alsike clover were seeded April 2, 2014 into 1 gallon pots prepared with a mix of 10% sand and 90% ready mix wetting compound. Plants were grown in Conviron climate controlled growth chambers. Initially the Conviron growth chambers were set to 100% light, 80% RH and a sixteen hour photophase at 18°C and an eighteen hour dark phase at 15°C. On 20-May-2014 a second seeding was done into the same soil mix.

Plants were watered roughly every two days or as pots became dry until 23-June-2014 when plants were watered twice a week or as pots became dry to facilitate flowering. Plants were first fertilized 9-May-2014. Subsequently, plants were fertilized with a formulation of

20% nitrogen 20% phosphorus and 20% potassium every two weeks. This fertilizer was mixed 1: 40 fertilizer: water and each pot was given 500 mL mix. To increase plant number and reduce crowding transplants were done 15-May-2014, 29-May-2014, 30-May-2014, 5-Jun-2014 and 9-Jun-2014.

On 21-May-2014 the growth chamber settings were changed to 20°C day temperature and 18°C night temperature to encourage flowering, and to simulate early summer temperatures in the Peace River region. To attempt to further stimulate flowering on 1-July-2014 growth room three was changed to 24°C day temperature with 18°C night temperature.

Unlike previous years, clover grown in the conviron chambers produced very few flowers and most plants did not produce flowers until late July and August, instead of in early June. The Conviron climate controlled growth chambers had been updated in the winter of 2013-2014 and the new lights may produce an inadequate light spectrum to facilitate flowering of legumes.

#### 2.6 HOST PLANT QUALITY TRIALS

Larvae were weighed with the case to prevent damaging or stressing the larvae. Case length was also measured. Each larva was then randomly either preserved in 95% ethanol or was separated to be reared. Not all larvae were included in the rearing experiment due to inadequate numbers of clover buds flowering in growth chambers. Larvae were reared individually to exclude any effects of competition (Gibbs et al. 2004) and to allow me to follow individual growth.

Larvae were reared on either on red clover flower heads or on alsike clover flower heads for the duration of each trial. Individual rearing chambers consisted of a 16 dram

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plastic snap cap vial (Figure 6A) held in place on a clover flower head with a foam plug with a slit cut through the middle (Figure 6B).



Figure 6. A. Coleophora deauratella larva inside 16 dram plastic rearing chamber. B. Individual plastic rearing chambers on alsike flower heads in Conviron environmentally controlled growth room.

Every week all larvae were removed from the growth rooms. Weights (including the case) of all the larvae as well as case length was recorded. Larvae that were dead, inactive, appeared dead or had caused significant damage to the foam plugs were preserved in 95% ethanol. Live, healthy larvae were returned to the growth rooms and given fresh clover flower heads.

Larvae were reared from the time they emerged until 20- August-2014. At that point all larvae exhibited signs of stress (excessive chewing and tunnelling into plugs) and all remaining larvae were weighed and preserved in 95% ethanol.

Preserved larvae were removed from cases under ethanol and larval length and head capsule diameter were measured to determine larval instar.

#### 2.7 STATISTICAL ANALYSIS

Statistical analyses were done in the open source program R 3.12 (R Core Development Team 2015). Larval density was calculated per flowerhead at each site. Total larval abundance was summarized per site and standard deviation was calculated. A  $\chi^2$ goodness of fit test was used to compare the ratio of larvae found in alsike clover plants and red clover plants.

Percent mortality was calculated for each plant species, and a  $\chi^2$  test of association was done to compare number of larvae that survived and died on red and alsike clover. All larvae that did not survive rearing were excluded from further rearing data analysis. Normality of larval weight data was proven with a Shapiro-Wilks test. The difference between the weights of the two rearing groups was determined with a parametric Welch Two Sample T-test.

#### 4. **RESULTS**

Two species of *Coleophora* moths were identified in the pheromone unitrap samples: *Coleophora mayrella* (10 individuals) and *Coleophora deauratella* (1067 individuals) (Figure 7). No *Coleophora trifolii* specimens were identified and no *Coleophora* females were found in the unitrap samples. A total of 78 subsamples (1067 individuals) were

#### examined.



Figure 7. Coleophora species identified in subsamples of 20 moths from pheromonebaited unitraps (n=1067). Scale is logarithmic.

No larvae were found in alsike flower heads collected from any of the sites. This was true for flower heads collected both from cultivated alsike clover fields and from volunteer plants growing along the field edge in the ditch (Table 1). *Coleophora* larvae were found at all sites, however, standard deviation between sites was large. The mean density per flowerhead was  $0.8 \pm 0.14$ . At site 2014-47 the density was 5.56 (Table 1). A total of 338 *Coleophora* larvae were collected. There was a statistically significant difference between the number of larvae collected from patches of red clover and alsike clover plants (n red =4, n alsike =4,  $\chi^2$ = 96.57, df = 1, P= < 2.2e-16 ).

			Approximate	Number	Density (Number
			Patch	of larvae	of larvae per
Site	Crop	Location	size(m <sup>2</sup> )	per patch	flower $\pm$ SEM )
Site 2014- 049	Red	Field	50	40	0.8±0.21
	Alsike	Ditch	15	0	$0\pm 0.00$
Site 2014-048	Red	Field	50	15	$0.3 \pm 0.08$
	Alsike	Ditch	10	0	0±0.00
Site 2014-046	Red	Field	50	5	0.1±0.05
	Alsike	Ditch	5	0	$0\pm 0.00$
Site 2014-047	Alsike	Field	10	278	5.56±0.82
	Red	Ditch	50	0	$0\pm 0.00$
Total	Red		160	338	0.8±0.14
	Alsike		80	0	$0\pm 0.00$

Table 1. Density of *Coleophora* sp. larvae in red and alsike flower heads (n=50 flowers/patch) sampled from collection sites and adjacent volunteer clover plants growing in the ditch on July 23, 2014. (Note: bold indicates cultivated clover crop.)

Larval mortality occurred for larvae reared both on alsike and red clover during the four week rearing period. Larvae reared on alsike clover (n=141) experienced 29% mortality (Figure 8) and larvae reared on red clover (n=20) experienced 5% mortality (Figure 8). Overall mortality of *C. deauratella* reared on red clover was significantly lower than mortality of larvae reared on alsike clover (n alsike =141, n red =20,  $\chi^2$ = 5.948, df = 1, P=0.019 Figure 8).



Feeding Bud type



Larval mortality did not occur at an even rate throughout the rearing period. Approximately 75% of larval mortality on alsike occurred during the first week of the experiment (Figure 9). The remaining 25% of mortality on alsike occurred evenly over the final weeks of the experiment (Figure 9). In the red clover rearing cohort there was no mortality until 1 larva died during the final week of the experiment (Figure 9).



Figure 9. Number of larvae surviving in each in rearing cohort over time. Rearing cohorts reared exclusively on red (solid line) or alsike (dashed line) clover for 4 weeks.

Overall, larvae reared exclusively on alsike clover gained significantly less weight (grams) than larvae reared exclusively on red clover (Figure 10). Larvae reared exclusively on alsike clover had a mean weight gain of  $0.00274 \pm 0.0001788$  grams ( $\bar{x}\pm$ SEM) (Figure 10) over the four week rearing period compared to larvae reared on red clover which experienced a significantly greater mean weight gain of  $0.00438 \pm 0.000721$  grams ( $\bar{x}\pm$ SEM) (Figure 10) over the four week rearing period (n red clover = 19, n alsike clover = 109, t = - 2.9093, p= 0.01407) (Figure 10).



Feeding Bud type

Figure 10. Overall weight change (grams) of larvae reared (4 weeks) exclusively on either alsike clover (n=109) or red clover (n=19). \* Denotes significant difference. (p=0.01407)

#### 5. **DISCUSSION**

The abundance of *C. deauratella* in clover fields was examined through collection of individual flower heads, as in Markkula and Myllymaki (1964). The data indicate a clear pattern in the abundance of *C. deauratella* larvae: larvae were found in red clover flower heads at all sites, and no larvae were present in any alsike flower heads from any sites.

The number of larvae collected from red clover flower heads varied between sites.

The density of larvae ranged from 0.1 larvae per flowerhead (n=50 flowerheads) to 5.56

larvae per flowerhead (n=50 flowerheads) (Table 1). In 2010 to 2012 average larval density in clover fields in the Peace River region (Mori et al. 2014) was lower than in this study in 2014. Densities in 2014 (Table 1) were also much higher than the 0.22 larvae per flowerhead found in samples collected from red clover pasture in Finland (Markkula et al. 1964). This is unsurprising because invasive species often may reach densities higher in their novel range than in their native range (Colautti et al. 2004) and this may be reflected at site 2014-47 where 5.56 larvae per red clover flowerhead was observed in flowers collected from the field edge and ditch. This could be because of *C. deauratella* larvae and/or eggs clustered on the small amount of red clover present. In Finland, *C. deauratella* numbers increased then decreased as the proportion of red clover decreased in the field (Markkula et al. 1964). Peak *C. deauratella* larval number was when 61-40% of the field was red clover, but decreased after that concentration (Markkula et al. 1964).

Previous research has shown male *C. deauratella* moth pheromone catch numbers are significantly correlated with larval abundance (Mori et al. 2014). However, when the results of pheromone trapping are compared with larval abundance documented, no correlations appear to emerge (J. Otani, unpublished data). The sites with the highest moth numbers are not the sites with the highest larval numbers. In fact the site that had the highest larval abundance (Table 1) also had the lowest moth abundance. This could be because what moths and larvae were in the field were densely congregated on the volunteer red clover in the field.

Since moth pheromone catch numbers typically relate to larval abundance, I analyzed moth pheromone trap species composition as a measure of larval species composition. *C. deauratella* larvae cannot be identified until the case is fully mature. Only 0.9% of moths

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captured were *C. mayrella*, and if 0.9% of larvae collected from the field were *C. mayrella* then potentially 3.13 larvae were not *C. deauratella*. This is important because *C. mayrella* is expected to thrive only on white clover, and any contamination with this species could hinder the results.

Larval growth was chosen as a measure of larval performance on the two different hosts. In their meta-review, Awmack and Leather found that between 1994 and 2000 the most used measure of insect performance was growth (30.2% of studies they examined) (Awmack and Leather 2002), and larval weight impacts overall fitness (Knolhoff and Heckel 2014).

Other studies have indicated that red clover is the primary host of *C. deauratella*, but the species has also been reported on alsike clover, stone clover, zig zag and white clover (Hammer 1937; Ellis and Bjørnson 1996). Based on this study, it is unclear whether *C. deauratella* in the Peace River region is able to feed and complete development on these potential other hosts. It is possible that observations of *C. deauratella* on these plants may have arisen from confusion between other Coleophorids like *C. mayrella* or *C. frischella*. It is also possible that these plants (within the same genus) may act as less preferred hosts in situations where red clover is not available. My study focused on the two species commonly grown for forage seed in the Peace River region. The sample sizes used in this experiment also limit the applicability of the results to the field. Most of the larvae were collected from a single site. There was also a bias in the number of flowers in the rearing treatments. This was because previous studies have shown very few or no larvae emerging from rearing on alsike and the population was biased to allow enough larvae to survive.

Future work should examine other indicators of insect performance, as well as female ovipositional preference. Current ecological theory supports the preference-performance theory: female insects will lay more eggs on plants that offspring perform well on, thereby increasing fitness (Thompson 1988; Thompson and Pellmyr 1991; Mayhew 1997; Gripenberg et al. 2010). While there has been plenty of supporting data for this theory, there have been examples of species that don't fit well into this theory, and examples of ecological factors (such as competitors, predators and abiotic factors) that modify the strength of the interaction between preference and performance (Thompson and Pellmyr 1991; Mayhew 1997). It would be interesting to see if the interaction between performance and preference for *C. deauratella* was coupled, as this species seems to have a distinct performance differences between two closely related hosts. *C. deauratella* larvae are relatively immobile until later instars, and Gripenburg et al. (2010) find in their meta-review that across taxa the coupling between performance and preference is especially strong in sessile or near-sessile larvae (Gripenberg et al. 2010).

A lack of co-evolutionary history can decouple preference and performance. Introduced *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae) moths oviposited on a wide range of novel cacti species, and in a ranked order contrary to larval survivorship (Jezorek et al. 2010). *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae), an invasive wood-boring beetle, displays a widely expanded host range in North America compared to its native range, however larvae appear to develop equally fine on all hosts (Morewood et al. 2003). The ecological ramifications of these introductions are unknown. Permanent host expansions and therefore increased herbivore pressure cannot be reliably predicted, especially without knowing which drives evolution: ovipositional choices or larval

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performance (Jezorek et al. 2010). Detrimental expansions may be driven by ovipositional cues common to historic and novel hosts. *C. cactorum* selects oviposition sites based on spine density and cladode number(Jezorek et al. 2010), while *Pieris oleracea* Harris (Lepidoptera: Pieridae) relies on secondary metabolites to select hosts, which prompts oviposition onto the less suitable host, garlic mustard(Keeler and Chew 2008). Manipulation of these hosts, which act as population sinks, in management of invasive insect herbivores is possible. While not practical for large scale management or eradication, this technique has strong potential to protect agricultural yield losses through trap cropping. This management strategy has the potential to decrease economic and ecological harm, while preventing release of toxic pesticides into the environment.

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