PROJECT DETAILS

- **Title**: Biocontrol of canola cutworms: Identification and attraction of parasitoids
- **Funders:** Alberta Canola and Manitoba Canola Growers
- **Research program:** Canola Agronomic Research Program (CARP)
- **Principal investigator:** Barbara Sharanowski, University of Manitoba
- **Collaborators/additional investigators:** Yvonne Lawley and Udari Wanigasekara
- **Year completed:** 2015

Final report

1. **Determine species and biology of parasitoids (through sampling, rearing, and dissection)**

Introduction:

Cutworms (Lepidoptera: Noctuidae) are economically important pests of several Canadian field crops, including canola, wheat, corn, and sunflower. Typically they feed on stems of young plants at or below the soil surface in the spring and may cause enough damage that a field needs to be reseeded. There are several species of economic importance in the Canadian prairies, including: the redbacked cutworm, *Euxoa ochrogaster* (Guenée); darksided cutworm, *E. messoria* (Harris); dingy cutworm, *Feltia jaculifera* (Guenée), and the army cutworm, *E. auxiliaries* (Grote). Chemical control is the only strategy currently used to control cutworms, but it is not highly effective as these caterpillars have a patchy distribution, are subterranean, and typically only emerge at night to feed. Therefore, natural enemies may play an important role by regulating cutworm populations. However, few studies have examined the potential for natural enemies to control cutworms and thus have been underutilized as biological control agents. This study examined the hymenopteran parasitoids community attacking economically important cutworms in Canada.

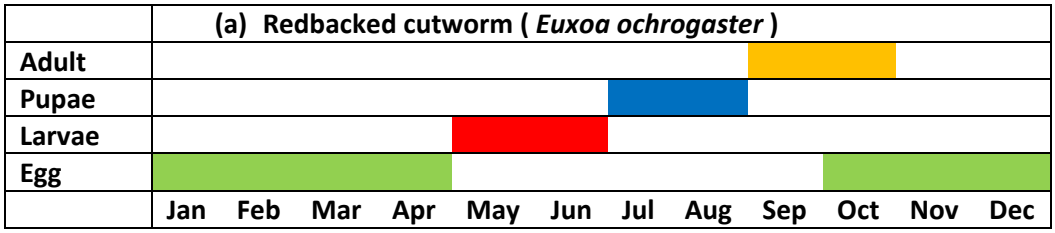
Materials and Methods:

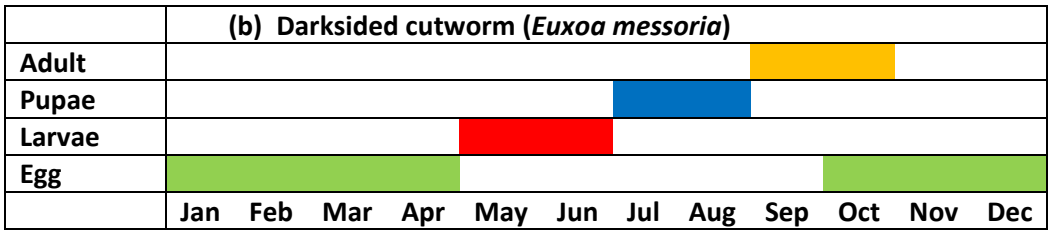
Cutworm samples were collected from agricultural crop fields in Manitoba from May to July 2012 and 2014, and reared parasitoids with their host cutworms were sent by collaborators from Alberta. Field collected cutworms were reared in the laboratory and provided artificial McMorran diet as a food source. All the unparasitized cutworms emerged as adult moths and parasitoids emerged from parasitized cutworms. Morphological identification of cutworms is difficult, when it has been parasitized. Therefore, we created a reference library of cytochrome oxidase I sequences obtained from identified adult moths. Currently we are comparing larval samples from parasitized larvae to these reference sequences to confidently identify the larval host to determine species-specific effects on the biology of parasitoids. Further, most of

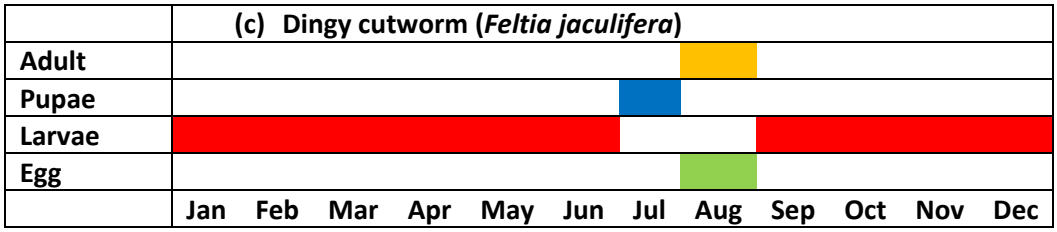
the parasitoid specimens were identified using taxonomic keys, and some of the specimens were sent to Canadian national Collection to further identification. This molecular work will be completed in end of 2015.

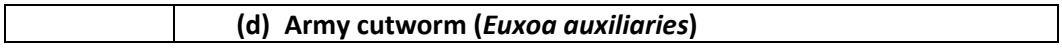
Results:

Compiling all the observations, we created life cycles for four major cutworm species in Canadian prairies (Figure 1). According to our results, the percentage of cutworms parasitized by hymenopterans were reduced in 2013 and 2014 relative to 2012 in Manitoba and it was lower in 2012 and 2014 compared to 2013 in Alberta (Table 1). From these hymenopteran parasitoids, 65% of them were in the family Encyrtidae, 21% of them were in the family Ichneumonidae and 14% of them were in the family Braconidae. We recorded two species from Encyrtidae, five species from Braconidae, and nine species from Ichneumonidae (Table 2). A user friendly taxonomic key to all observed parasitoid species will be developed at the end of 2015. Further, we are planning to finish this manuscript in early 2016.









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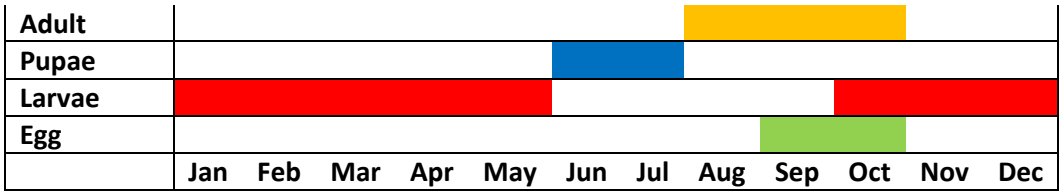

Figure 1: Generalized life cycles for four major cutworm species found in Canadian prairies. (a) *Euxoa ochrogaster,* (b) *Euxoa messoria,* (c) *Feltia jaculifera,* and (d) *Euxoa auxiliaries*.

Table 1: Percentage of parasitized cutworms in Manitoba and Alberta from 2012 to 2014

	2012	**2013**	**2014**
Manitoba	6.8%	5.3%	2.8%
Alberta	10.5%	26%	16.3%

Adult parasitoid emergence periods differed depending on the species and the locality. The emergence time is critical for offering cover crops at the right time to enhance the effectiveness of parasitoids (Table 2). During the study period we found that *Copidosoma* spp. are the most common parasitoids in both Alberta and Manitoba. Further, we found that *C. cuproviridis* emerges earlier than *C. bakeri*, but their parasitism rates seem to be very low. Therefore, we identified and characterized potential cover crops that can attract, maintain, and enhance *C. cuproviridis* in the ecosystem.

Table 2: Species and phenology of hymenopteran parasitoids attacking four different cutworm species found in

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Canadian Prairies

Family	Subfamily	Valid Name		Synonyms	Locality	Host	Crop	Length of Life cycle (days)	Adult emergence time	Biological Remarks
		Genus	Species							
Encyrtidae	Encyrtinae	*Copidosoma*	*bakeri*	*Berecyntus bakeri Berecyntus bakeri arizonensis Berecyntus bakeri euxoae Berecyntus bakeri gemma Copidosoma bakeri gemma Litomastix bakeri*	AB, MB	*Euxoa ochrogaster Euxoa messoria Euxoa auxiliaries Feltia jaculifera*	Canola, Wheat, Corn, Pumpkin, Soybean, Barley	60-70 MB 40-50 AB	August to September in MB and June to July in AB	Polyembryonic endoparasitoid (koinobiont)
	Encyrtinae	*Copidosoma*	*cuproviridis*		AB, MB	*Euxoa auxiliaries*	Wheat	40-50 MB 35-45 AB	Mid July in MB and mid June in AB	Polyembryonic endoparasitoid (koinobiont)
Braconidae	Microgastrinae	*Cotesia*	spp.	*Cryptapanteles, Protapanteles, Stenopleura*	AB	*Euxoa auxiliaries*	Canola, wheat and canola stubble	19-21	May to June	Gregarious endoparasitoids (koinobiont)
	Meteorinae	*Meteorus*	sp. I	*Pachythecus, Protelus, Saprotichus, Zele, Zemiotes*	AB		Peas on canola and weed fallow	55-60	July	Gregarious endoparasitoids (koinobiont)
		Meteorus	sp. II	*Pachythecus, Protelus, Saprotichus, Zele, Zemiotes*	AB		Peas on canola and weed fallow	18-20	June	Gregarious endoparasitoids (koinobiont)
	Microgastrinae	*Microplitis*	*kewleyi*		MB	*Euxoa ochrogaster*	Canola	20-25	July	Gregarious endoparasitoids (koinobiont)
	Microgastrinae	*Parotapanteles (Sathon)*	neomexicanus	*Apanteles caudatus*	AB			30-35		Gregarious endoparasitoids

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Family	SubFamily	Valid Name		Synonyms	Locality	Host	Crop	Length of Life cycle (days)	Adult emerging time	Remarks
		Genus	Species							
Ichneumonidae	Ichneumoninae	*Diphyus*	*euxoae*	*Diphyus orientis Ichnumon variegatus*	AB	*Euxoa auxiliaries*	Winter Killed 1st year alfalfa, Peas on canola and weed fallow	35-40	Early June to Mid July	Solitary endoparasitoid (koinobiont)
	Ichneumoninae	*Ichneumon*	sp. I		AB	*Euxoa auxiliaries*	Barley, Canola, Winter Killed 1st year alfalfa, Peas on canola and weed fallow	35-40	Early June	Solitary endoparasitoid (koinobiont)
	Ichneumoninae	*Spilichneumon*	*superba*	*Ichnumon Koebeli*	AB	*Euxoa auxiliaries*	Canola, wheat and canola stubble, Peas on canola and weed fallow	35-40	Early June to early July	Solitary endoparasitoid (koinobiont)
	Ichneumoninae	*Ichneumon*	sp. II		AB	*Euxoa auxiliaries*	Oats, Wheat and canola stubble, Peas on canola and weed fallow	35-40	Early July	Solitary endoparasitoid (koinobiont)

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Family	Subfamily	Valid Name		Synonyms	Locality	Host	Crop	Length of Life cycle (days)	Adult emerging time	Remarks
		Genus	Species							
Ichneumonidae cont'd.	Campopleginae	*Campoplex*	sp. I	*Campoplegina, Lathroples, Phaedroctonus, Sinophrus, Dioratica, Omorga, Pseuderipternoids, Zatranosema, Eulimneria, Omorgus, Sesioplex*	AB			30-35	June	Solitary endoparasitoid (koinobiont)
	Campopleginae	*Campoplex*	sp.II	*Campoplegina, Lathroples, Phaedroctonus, Sinophrus, Dioratica, Omorga, Pseuderipternoids, Zatranosema, Eulimneria, Omorgus, Sesioplex*	MB			35-40	August	Solitary endoparasitoid (koinobiont)
	Tryphoninae	*Netelia*	*ocellata*	*Paniscus immaculatus, Paniscus microocellatus*	MB	*Euxoa auxiliaries*	Wheat	55-60	Early August	Solitary endoparasitoid (koinobiont)
	Banchinae	*Exetastes*	*syriacus*	*Exetastes ruficoxalis*	AB		Red clover	70-75	Early August	*Solitary endoparasitoid (koinobiont)*
	Anomalonine	*Erigorgus*	sp	*Barylypa, Camposcopus, Kokujewiella, Nenethes, Paranomalon, Sympratis*	AB	*Euxoa auxiliaries*	Alfalfa	100-120		Solitary endoparasitoid (koinobiont)

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2. Characterizing flowering cover crop species for potential use in conservation biocontrol

Introduction

The objective of this activity is to identify and characterize plant species that could be used as cover crops to attract, maintain, and enhance parasitoids. Once identified, these cover crops could be used to develop a habitat management strategy for cutworm control.

Materials and Methods

Field experiments were conducted during the 2013 and 2014 growing seasons to evaluate the flowering periods and characteristics of plant species that are currently grown as cover crops or show potential for use as cover crops. These experiments were conducted at Ian N. Morrison Research Farm in Carman, MB, and at the Canada Manitoba Crop Diversification Center in Portage La Prairie, MB. The experiments were set up with a randomized complete block design and four replicates. Plot size was 2 m wide by 8 m long.

Eleven plant species were evaluated as cover crops and canola was included in the trail as a reference crop. The plant species are named in Table 1 along with their lifecycle, and plant family. Three cover crop mixtures were created from this list of eleven species based on flower color. The “purple” mixture included flax, phacelia, hairy vetch. Chickling vetch was added to the purple mixture in 2014 The “yellow” mixture included brown mustard, camelina, canola, and wild mustard. The “white” mixture included berseem clover, buckwheat, field pennycress, and tillage radish. The mixtures were only tested at Carman due to space restrictions at Portage. The experiments were seeded in mid to late May at Carman and in early June in Portage. Plots were hand weeded as required. Detailed notes were taken to record flower color, the first and last day of flowering, 50% flowering, and 90% of plants finished flowering for each plant species grown alone and when grown in mixture. In 2014, the date the 50% of plants were budding was also noted. Average dates were calculated based on the four replicates for each species within each site year. The duration of flowering was calculated based on the first and last days of flowering. The day of first killing frost was used in place of the last day of flowering for plant species that continued to flower into the fall.

Results:When did the cover crops start to flower?

Plants could be characterized as those that flower early in the growing season and those that flower later in the growing season (Tables 4-7). Looking at each plant species grown in monoculture we observed that early flowering plant species were mostly in the *Brassica* family and included: wild mustard, field pennycress, brown mustard, canola, tillage radish, and buckwheat. Later flowering species included: camelina, phacelia, hairy vetch, chickling vetch and berseem clover.

How long did the cover crops flower?

Many of the cover crops species that flowered earliest also flowered for a relatively short period of time (Tables 4-7). This group included field pennycress and wild mustard. Some cover crop species that flowered later also flowered for a longer period of time, for example phaselia and hairy vetch. Some plant species flowered for extended periods of time that were terminated only by the first fall frost. Buckwheat was exceptional as it flowered early and continued to flower for a long period of time.

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Did growing the cover crops in mixtures change flowering period?
Growing these cover crops species in mixtures influenced the flowering period of individual species. When comparing first flowering date of all cover crop species when grown in mixtures compared to when grown as a single species monoculture for all four site years, we observed that growing them in mixtures resulted in earlier flowering (Figure 2). Growing plants in mixtures also resulted in a longer duration of flowering for individual species (Figure 3).

Growing plants in mixtures also increased the overall flowering period duration by overlapping the complimentary flowering periods of individual species. The white and purple flowering mixtures had the longest flowering periods compared to the yellow mixture in both years (Table 7 and 8). The yellow mixture had the shortest flowering period. This yellow mixture was made up entirely of plants in the Brassica family, while the white and purple mixtures had plants from more than one plant family. The buckwheat and berseem clover in the white mix and the phacelia and vetch in the purple mix helped to extend the duration of flowering for these mixtures. The cover crop mixtures selected for this study were not designed to extend flowering period. With the information learned in this study about the relative flowing periods and durations for each species and the observation that growing cover crops as mixtures extends flowering period, it would be possible to design cover crop mixtures for this purpose.

Table 3. Characteristics of plant species tested as potential cover crops during the 2013 & 2014 growing season.

Common name	Scientific name	Flower color	Life cycle	Plant family
Berseem clover	*Trifolium alexandrinum*	White	Summer Annual	Fabaceae
Brown mustard	*Brassica juncea* (L.) Czern.	Yellow	Summer Annual	Brassicaceae
Buckwheat	*Fagopyrum esculentum*	White	Summer Annual	Polygonaceae
Camelina	*Camelina sativa* (L.) Crantz.	Pale yellow	Summer or Winter Annual	Brassicaceae
Canola	*Brassica napus* L.	Yellow	Summer Annual	Brassicaceae
Chickling vetch	*Lathyrus sativus*	White, pink, or purple	Summer Annual	Fabaceae
Field pennycress	*Thalaspi arvense* L.	White	Summer or Winter Annual	Brassicaceae
Flax	*Linum usitatissiumum* L.	Light purple	Summer Annual	Linaceae
Hairy vetch	*Vicia villosa* Roth.	Dark purple	Winter Annual	Fabaceae
Phacelia	*Phacelia tanacetifolia* Benth.	Light purple	Summer Annual	Hydrophyllaceae
Tillage radish	*Raphanus sativus* L.	White and light purple	Summer Annual	Brassicaceae
Wild mustard	*Sinapus arvensis* L.	Yellow	Summer Annual	Brassicaceae

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Table 4. Flowering dates and durations for plant species tested as potential cover crops, grown in monoculture, at Carman, MB during the 2013 growing season.

Cover Crop	First day of flowering	50% or more of plants flowering	90% of plants finished flowering	Finished flowering	Duration of flowering
	---------- Calendar Day ----------				# Days
Berseem clover	31-Jul	28-Aug	16-Sep	16-Sep	47
Brown mustard	27-Jun	01-Jul	24-Jul	10-Aug	44
Buckwheat	02-Jul	02-Jul	05-Jul	16-Sep	81
Camelina	03-Jul	06-Jul	21-Jul	24-Jul	21
Canola	28-Jun	03-Jul	22-Jul	01-Aug	34
Field pennycress	26-Jun	28-Jun	16-Jul	21-Jul	25
Flax	06-Jul	10-Jul	24-Jul	30-Jul	24
Hairy vetch	10-Jul	*	*	16-Oct	98
Phacelia	06-Jul	12-Jul	21-Aug	04-Sep	60
Tillage radish	28-Jun	03-Jul	05-Aug	19-Aug	52
Wild mustard	26-Jun	28-Jun	02-Jul	12-Aug	48

Notes: Experiment was planted at Carman on May 23, 2013. First killing frost was on October 14, 2013.
* Hairy vetch continued to flower until first killing frost, but did not reach 50% or 90% bloom.

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Table 5. Flowering dates and durations for plant species tested as potential cover crops, grown in monoculture, at Carman, MB during the 2014 growing season.

Cover Crop	Budding started for 50% of plants	First day of flowering	50% or more of plants flowering	90% of plants finished flowering	Finished flowering	Duration of flowering
	---------- Calendar Date ----------					# Days
Berseem clover	05-Aug	28-Jul	09-Aug	*	12-Sep	46
Brown mustard	21-Jun	25-Jun	03-Jul	03-Aug	10-Aug	45
Buckwheat	17-Jun	20-Jun	28-Jun	12-Sep	15-Sep	87
Camelina	21-Jun	23-Jun	28-Jun	12-Jul	25-Jul	32
Canola	18-Jun	23-Jun	28-Jun	21-Jul	28-Jul	35
Chickling vetch	29-Jun	30-Jun	06-Jul	03-Aug	12-Aug	43
Field pennycress	15-Jun	16-Jun	19-Jun	09-Jul	15-Jul	29
Flax	02-Jul	02-Jul	07-Jul	22-Jul	04-Aug	33
Hairy vetch	04-Jul	08-Jul	02-Aug	23-Aug	12-Sep	66
Phacelia	25-Jun	02-Jul	05-Jul	09-Aug	14-Aug	44
Tillage radish	19-Jun	24-Jun	30-Jun	16-Aug	21-Aug	58
Wild mustard	17-Jun	18-Jun	21-Jun	24-Jul	28-Jul	40

Notes: Experiment planted at Carman May 14, 2014. First killing frost was on September 12, 2014. Hairy vetch continued to flower until killing frost.*Berseem clover continued to flower until first killing frost, but did not reach 90% bloom.

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Table 6. Flowering dates and durations for plant species tested as potential cover crops, grown in monoculture, at Portage la Prairie, MB during the 2013 growing season.

Cover Crop	First day of flowering	50% or more of plants flowering	90% of plants finished flowering	Finished flowering	Duration of flowering
	---------- Julian Day ----------				# Days
Berseem clover	14-Aug	26-Aug	^	14-Oct	62
Buckwheat	05-Jul	10-Jul	07-Sep	18-Sep	75
Camelina	10-Jul	12-Jul	02-Aug	08-Aug	29
Canola	10-Jul	17-Jul	11-Aug	20-Aug	41
Hairy vetch	03-Aug	*	*	14-Oct	72
Phacelia	22-Jul	01-Aug	03-Sep	18-Sep	58
Tillage radish	10-Jul	17-Jul	20-Aug	26-Aug	48
Wild mustard	04-Jul	08-Jul	15-Aug	22-Aug	48

Notes:Experiment was planted at Portage on June 7, 2013. First killing frost was on October 14, 2013.
^ Berseem clover continued to flower until first killing frost, but did not reach 90% bloom.
* Hairy vetch continued to flower until first killing frost, but did not reach 50% or 90% bloom.

Table 7. Flowering dates and durations for plant species tested as potential cover crops, grown in monoculture, at Portage la Prairie, MB during the 2014 growing season.

Cover Crop	Budding started for 50% of plants	First day of flowering	50% or more of plants flowering	90% of plants finished flowering	Finished flowering	Duration of flowering
	---------- Calendar Date ----------					# Days
Berseem clover	24-Aug	22-Aug	03-Sep	*	09-Oct	48
Buckwheat	15-Jul	14-Jul	21-Jul	26-Sep	09-Oct	87
Camelina	14-Jul	18-Jul	23-Jul	09-Aug	13-Aug	26
Canola	15-Jul	16-Jul	22-Jul	11-Aug	17-Aug	32
Hairy vetch	30-Jul	29-Jul	20-Aug	02-Sep	09-Oct	72
Phacelia	27-Jul	27-Jul	06-Aug	25-Sep	09-Oct	75
Tillage radish	17-Jul	16-Jul	23-Jul	26-Aug	08-Sep	55
Wild mustard	14-Jul	14-Jul	17-Jul	17-Aug	21-Aug	38

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Notes: Experiment was planted at Portage June 10, 2014. First killing frost was October 9, 2014.

Tillage radish, berseem clover, hairy vetch and buckwheat began flowering before 50% of the crop was budding. *Berseem clover never reached 90% finished flowering before fall frost.

Table 8. Flowering dates and durations for plant species tested as potential cover crops, grown in mixes, at Carman, MB during the 2013 growing season.

Cover Crop	First day of flowering	50% or more of plants flowering	90% of plants finished flowering	Finished flowering	Duration of flowering
	---------- Calendar Day ----------				# Days
White mixture	**10-Jul**			**16-Sep**	**80**
Berseem clover	25-Aug	23-Aug	11-Sep	16-Sep	23
Buckwheat	27-Jun	2-Jul	25-Aug	16-Sep	81
Field pennycress	10-Jul	27-Jun	9-Jul	14-Jul	4
Radish	28-Jun	1-Jul	11-Aug	19-Aug	52
Yellow mixture	**26-Jun**			**3-Aug**	**38**
Brown mustard	27-Jun	6-Jul	21-Jul	3-Aug	36
Camelina	3-Jul	5-Jul	17-Jul	19-Jul	16
Canola	28-Jun	1-Jul	21-Jul	26-Jul	28
Wild Mustard	26-Jun	28-Jun	22-Jul	1-Aug	36
Purple mixture	**6-Jul**			**31-Aug**	**72**
Flax	6-Jul	9-Jul	19-Jul	22-Jul	16
Phacelia	7-Jul	13-Jul	26-Aug	31-Aug	55
Vetch	11-Jul	10-Aug	13-Sep	16-Sep	68

Notes: Experiment was planted at Carman on May 23, 2013. First killing frost was on October 14, 2013.

Table 9. Flowering dates and durations for plant species tested as potential cover crops, grown in mixes, at Carman, MB during the 2014 growing season.

Cover Crop	Budding started for 50% of plants	First day of flowering	50% or more of plants flowering	90% of plants finished flowering	Finished flowering	Duration of flowering
	---------- Calendar Date ----------					# Days
White mixture	**15-Jun**				**15-Sep**	**92**
Berseem clover	6-Aug	6-Aug	10-Aug	29-Aug	12-Sep	37
Buckwheat	17-Jun	20-Jun	30-Jun	12-Sep	15-Sep	87
Field Pennycress	15-Jun	16-Jun	19-Jun	9-Jul	14-Jul	28
Tillage radish	19-Jun	23-Jun	30-Jun	28-Jul	18-Aug	56
Yellow mixture	**17-Jun**				**4-Aug**	**48**
Brown mustard	28-Jun	30-Jun	4-Jul	29-Jul	4-Aug	36
Camelina	22-Jun	26-Jun	30-Jun	10-Jul	18-Jul	22
Canola	18-Jun	24-Jun	28-Jun	23-Jul	28-Jul	34
Wild Mustard	17-Jun	19-Jun	22-Jun	25-Jul	2-Aug	45
Purple mixture	**24-Jun**				**29-Aug**	**62**
Chickling Vetch	1-Jul	2-Jul	6-Jul	4-Aug	12-Aug	41
Flax	1-Jul	2-Jul	7-Jul	25-Jul	30-Jul	28
Hairy Vetch	3-Jul	7-Jul	10-Aug	20-Aug	29-Aug	54
Phacelia	24-Jun	2-Jul	7-Jul	7-Aug	13-Aug	42

Notes: Experiment planted at Carman May 14, 2014. First killing frost was on September 12, 2014.

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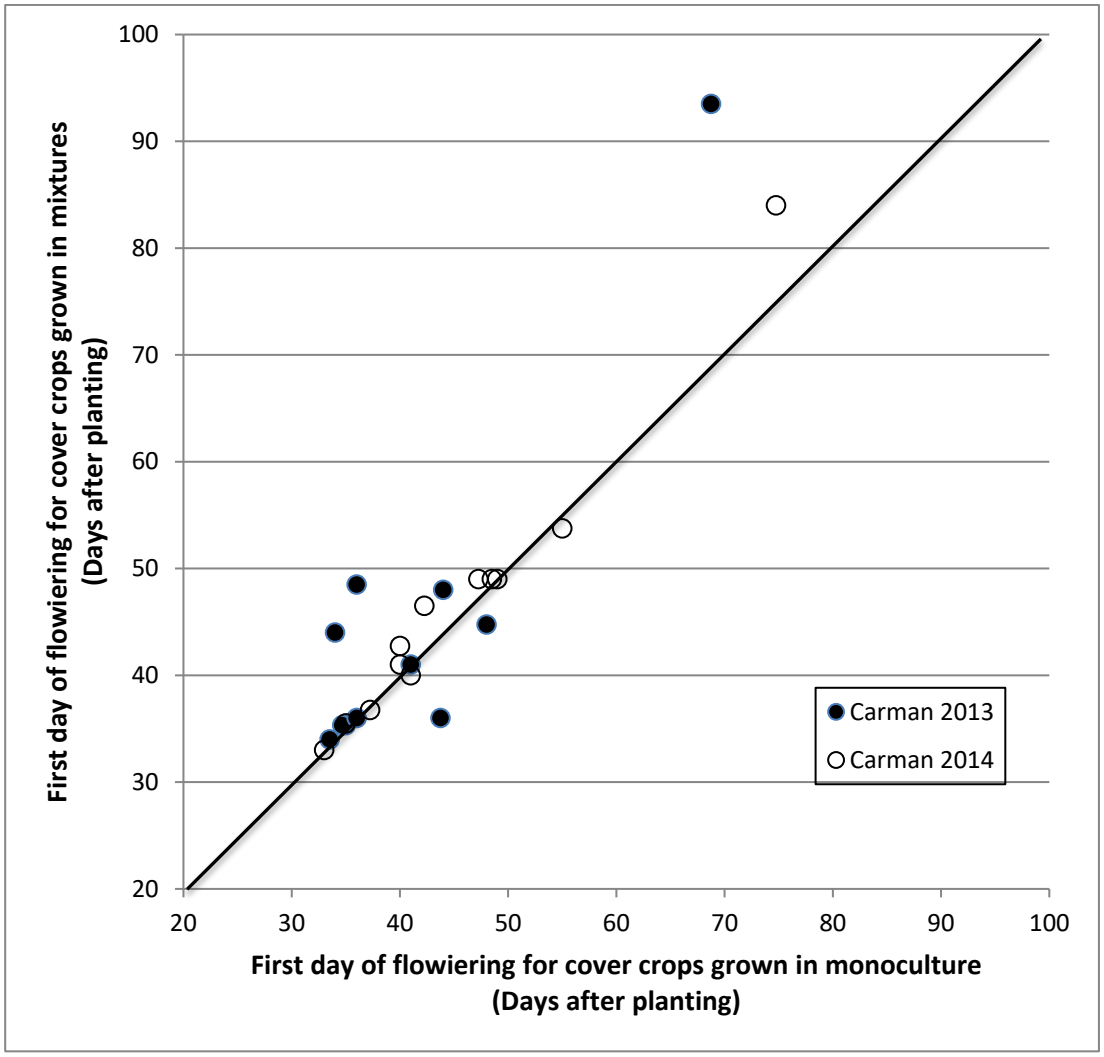

Figure 2: Comparison of first day of flowering for cover crops grown in mixtures compared to monoculture at Carman, MB in 2013 and 2014. Each data point represents the average of four reps for one cover crops species at one site year.

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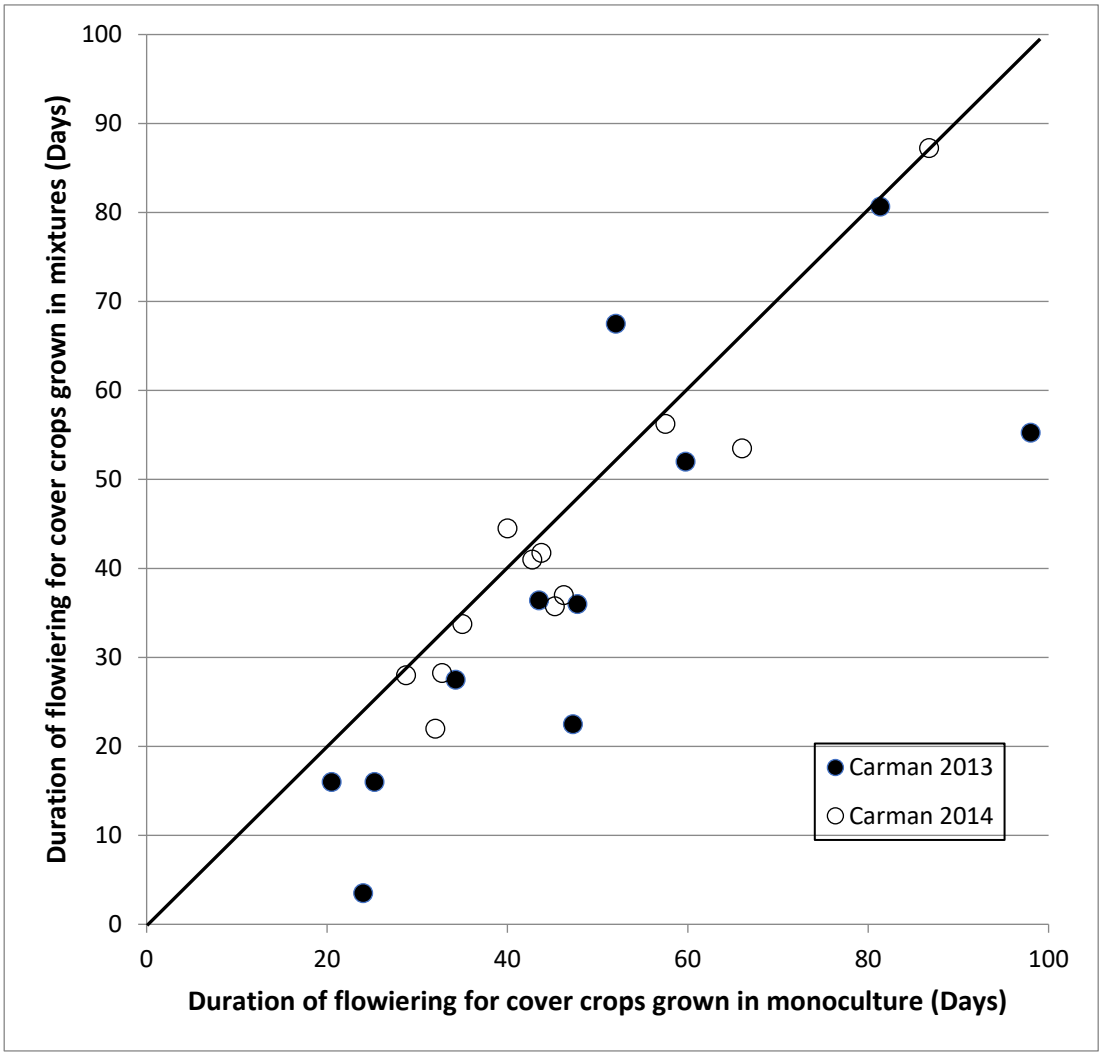

Figure 3: Comparison of flowering duration for cover crops grown in mixtures compared to monoculture. Each data point represents the average of four reps for one cover crops species at one site year.

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3. **Correlating reproductive period of main parasitoid with prospective cover crops (choice tests with parasitoids and flowers)**

Introduction

Research on parasitoids attacking cutworms in Canada has been limited and parasitoid preferences for flowering plants has not been tested in this system. Although there are several species of parasitoids attacking cutworms, most have very low parasitism rates in the field. Parasitoids often depend on additional food sources such as pollen, nectar, and extra floral nectaries to fulfill their nutritional requirements, enhance life expectancy, increase fecundity, gain energy for flight, and increase percent parasitism.
In here, we chose polyembryonic egg-larval parasitoid *Copidosoma cuproviridis*, as it is one of the most common parasitoid wasps in Canadian prairies. They are proovigenic parasitoids with finite egg load, as a benefit of increased longevity they have sufficient time to deposit eggs, especially if hosts are patchy or scarce. Thus, enhancing life expectancy is an important aspect of additional nutritional resources for parasitoid wasps. Cover crops have the potential to increase landscape biodiversity while providing significant benefits to the ecosystem and economic returns.

Materials and Methods:

Field Collection and Rearing

Cutworm samples were collected from infested field crops in Manitoba from May to July 2013. Infested crops were discovered through communication with provincial extension agents with Manitoba Agriculture, Food and Rural Development (MAFRD). Since cutworms typically have a patchy distribution (Ayre and Lamb 1990), soil samples were checked only from infested zones within fields. Soil samples were examined for cutworm larvae by digging a 2 cm radius circle around a damaged plant. All immature larval stages available were hand-collected. Following the methods outlined in Shaw (1997), cutworms were individually reared in the laboratory in labeled transparent plastic cups (3.5 ml) and provided McMorran diet as a food source (Bucher and Bracken 1976; McMorran 1965).

Upon adult emergence of *C. cuproviridis*, approximately 500 wasps were selected randomly per host and divided into two treatments: (1) food experienced treatment (provided with diluted honey (20% honey in distilled water)); and (2) food inexperienced treatment (provided with only water). For the longevity test, an additional treatment was established where approximately 100 wasps were randomly selected but not given water or honey (food and water inexperienced control). Only female wasps were used for the experiments.

Selection of plants

Nine plant species were chosen a priori based on flowering period to coincide with adult wasp emergence and flower colour (Table 1). Seeds from these nine species were planted in individual pots with peat soil (Sun Gro horticulture, Canada, 60-70% sphagnum peat moss, horticulture perlite and dolomite lime) and plants were watered twice a week. Plants were grown until they flowered in a walk-in growth chamber at 22 ±2°C; 60 ± 10% RH, and a 16L:8D photoperiod.

Plant preference experiment

To determine the most attractive plant species to *C. cuproviridis*, one pot for each of the nine plant species (in flower) was placed inside a 2×2×1 m cage covered by fine mesh and arranged in a circle. Two hundred randomly selected food inexperienced (between 24 and 72h old) parasitoids were released in the center of the cage and allowed to settle for 30 minutes. Then the number of parasitoids found on each flowering plant was recorded by using a hand magnifying lens to visualize the wasps. This experiment was repeated twice using wasps emerged from two different hosts for a total of 800 wasps including 200 food inexperienced and 200 food experienced from each host. All plants and parasitoids were removed and the cage cleaned after each trial. Different flowers in new pots were used for each new trial and the flower pots were relocated to the opposite side of the cage to change the relative position of the flower species to prevent any bias due to location. Flower preference (number of wasps settling on each flower) was analyzed with a split-plot analysis of variance with feeding treatment (food experienced vs. food inexperienced wasps) as the whole-plot factor, flower species (nine species) as the sub-plot factors, and mother (two cohorts) as a blocking factor. Significant interactions were explored within main effects with single factor ANOVAs. Comparisons among flower treatments within main effects were conducted using pairwise comparisons adjusted for multiple comparisons by the sequential Bonferroni method (Rice 1989). Residual plots indicated that data fitted the assumptions of this analysis without a transformation. All statistical tests in this and the following sections were performed using R (R Development Core Team, 2011). Based on these studies, the four most attractive plants to *C. cuproviridis* (buckwheat, camelina, canola and mustard) were used for all other experiments.

Colour choice experiments

Dual choice tests were carried out to determine whether food inexperienced and food experienced *C. cuproviridis* specimens have a preference for a specific flower colour. Coloured paper sheets matching the floral colour were used as visual stimuli to avoid any floral size and shape effect on choice. The Gardner's colour wheel (by Sydney Eddison, The colour Wheel Company) was used as a standard to describe floral colours (Table 1). Only the colours of the four most attractive flowers (buckwheat, camelina, canola and mustard) determined by the previously described multiple choice experiment were tested. Buckwheat and camelina floral colours were referred to as white and yellow 1, respectively, throughout the study. As canola and mustard have a very similar bright yellow colour, they were referred to as yellow 2. Then dual choice tests were carried out by placing two different colour discs (2.5 cm diameter) at two corners on the same side of a square plexi-glass box (165 mm x 165 mm x 95 mm). The colour paper discs were fixed on the inside of the box and replaced after every trial. The positions of the two coloured discs were randomly alternated and the box was cleaned after each individual test to remove any potential odour cues. The box was illuminated with two 35W white LED bulbs covered with a sheet of white filter paper to dim and diffuse the light, following the methods of Lucchetta *et al*. (2008). Then a parasitoid wasp was inserted into the plexi-glass box using a small aspirator and time was recorded as soon as the wasp was introduced, following Wäckers (1994). Each dual choice comparison had 30 replicates. When a wasp was observed on one edge of the box (near or on) for at least 10 consecutive seconds, this was considered as a choice. Wasps that did not make a choice during the 5 min observation period were considered as "indecisive" and excluded from the statistical analysis following the methods outlined by Lucchetta *et al*. (2008). Each flower colour was first tested against green

to examine whether parasitoids were attracted preferentially to a floral colour or potentially to the green leaves of a plant. A generalized linear model with a Poisson distribution was conducted to test the effects of plant species, feeding status (food experienced or food inexperienced) and host mother on the settling response of wasps. We performed an analysis of deviance test to evaluate the contribution of each variable to explain the data (Venables and Ripley 2002). All interaction terms were included initially in the model, but only significant terms were retained in final models. Significant or marginally significant food and colour interactions were explored within main effects with single factor models.

Odour choice experiments

For these dual choice comparisons, flowers were collected before noon and tested shortly thereafter as flower fragrance can decrease throughout the day (Tollsten 2008). After cutting, the floral stalk was wrapped in wet cotton to prevent wilting. Both food experienced and inexperienced parasitoids that were 24 to 36 hours old were used for the experiment. Flowers were placed into an Erlenmeyer flask (25 ml) and wrapped with a piece of black cloth to prevent visual cues. For each test, two flasks with different flower species were placed 22 cm apart from each other on a round rotating table which was placed inside a cage (1×1×1 m). Following the protocol outlined in Wäckers (1994), one parasitoid wasp was placed into the cage using a small aspirator and time was counted as soon as the wasp was inserted. Parasitoids were tested individually for five minutes. A choice was deemed when the wasp landed on one of the flasks. Wasps that did not make a choice during the five minute observation period were deemed indecisive and excluded from the statistical analysis following Lucchetta *et al*. (2008). Four different flower odours were compared in separate dual choice tests, resulting in a total of six comparisons replicated 30 times. Chi-square tests were conducted to determine if food experiences and food inexperienced parasitoids were significantly attracted to a specific floral odour over the others.

Longevity experiment

To investigate female longevity under different nutritional regimes, the four most attractive plant species (mustard, camelina, canola, and buckwheat) determined from the multiple-choice test described above were selected for the longevity test. Flowers from these species were cut and transferred into small glass vials (15 ml) while their stems were under water to prevent air trapping in capillaries (Fig. 1). Seven treatments were established including: 4 floral nectar treatments (a flower species plus water); honey treatment (honey plus water); water only; and a negative control with no food or water source (control). Flower bunches with bloomed flowers were selected and flowers were changed every third day.

Newly emerged adult *C. cuproviridis* were kept without food for a period of 24 hours before the start of each experiment and groups of 10 randomly selected wasps were introduced in each arena for each test. These studies were carried out under controlled conditions in a growth room (24C, 16L:8D). Observations were made daily and mortality was recorded. To determine whether the life expectancy of *C. cuproviridis* was significantly enhanced by feeding on a specific flowering plant over the others and versus the controls, the Kaplan–Meier survival function was used to estimate survival curves and the Cox proportional hazards model was performed to compare them statistically (Crawley, 2012). A significant overall model was further explored with pairwise

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comparisons using the Cox proportional hazards model adjusted by the sequential Bonferroni method for multiple comparisons (Rice 1989).

Results:

Dual choice tests were carried out to determine whether food inexperienced and food experienced *C. cuproviridis* specimens have a preference for a specific flower colour and floral odour. The colour choice test results suggested that yellow is much more attractive than white and green for both fed and starved wasps (Figure 5), and odour test results demonstrated that food inexperienced *C. cuproviridis* significantly favored brassicaceae floral odours (Figure 6). Further, canola, camelina, mustard and buck wheat were used to investigate female longevity under different nutritional regimes and found survival time of parasitoids on canola, camelina, mustard and buck wheat were similar to each other, but reduced relative to honey (Figures 7). Regardless, the additional nutritional resources provided by the tested cover crops did improve longevity and thus can maximize the efficiency of the parasitoid's ability to lay eggs. We are planning to repeat these experiments in summer 2015 and manuscript from these results will be submitted in fall 2015.

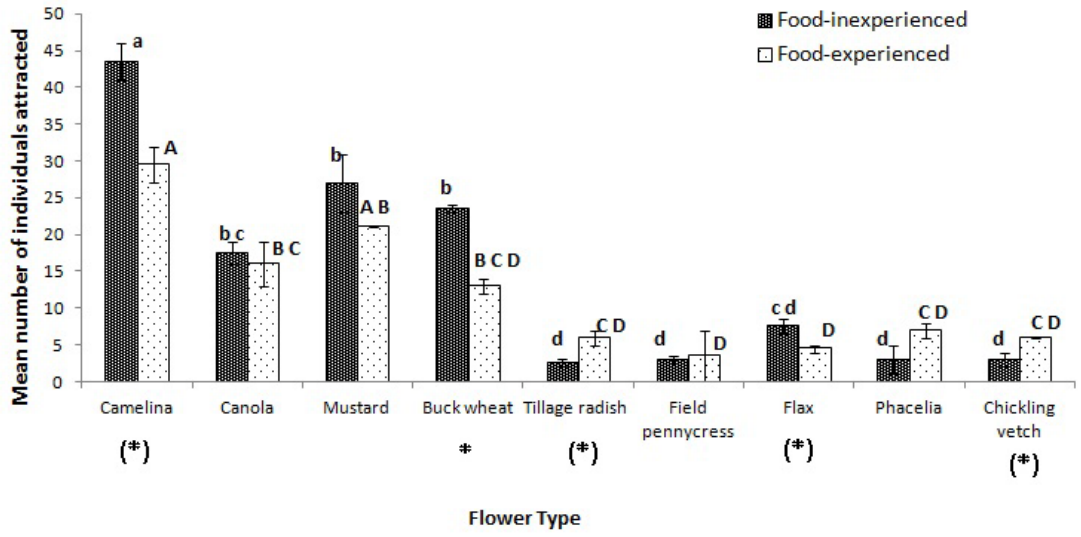

Figure 4: Plant preference experiment. Mean (± SE) number of food experienced and inexperienced *C. cuproviridis* attracted to different flowering plants (n=400 per feeding status). Statistical differences in preferences are shown via lowercase and uppercase letters for food inexperienced and food experienced wasps, respectively (pairwise comparisons adjusted for multiple comparisons by the sequential Bonferroni method, $P < 0.05$). Asterisks below flower names refers to tests of feeding condition within floral regimes, where * indicates $P < 0.05$ and (*) indicates $P < 0.10$.

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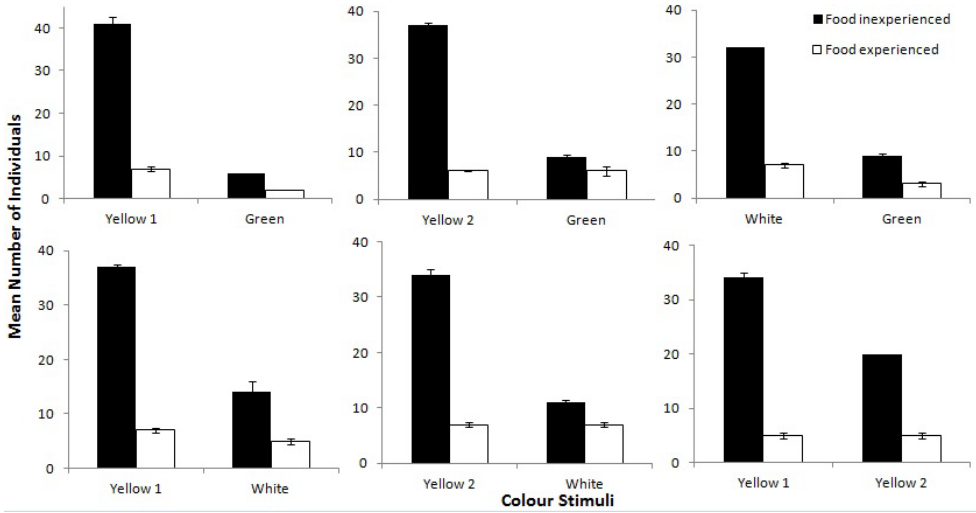

Figure 5: Mean (±SE) number of food experienced and food inexperienced C. cuproviridis attracted to color stimuli offered in a dual choice test (n= 30 for each experiment): a) yellow 1 vs. green b) yellow 2 vs. green; c) white vs. green; d) yellow 1 vs. white; e) yellow 2 vs. white; and f) yellow 1 vs. yellow 2.

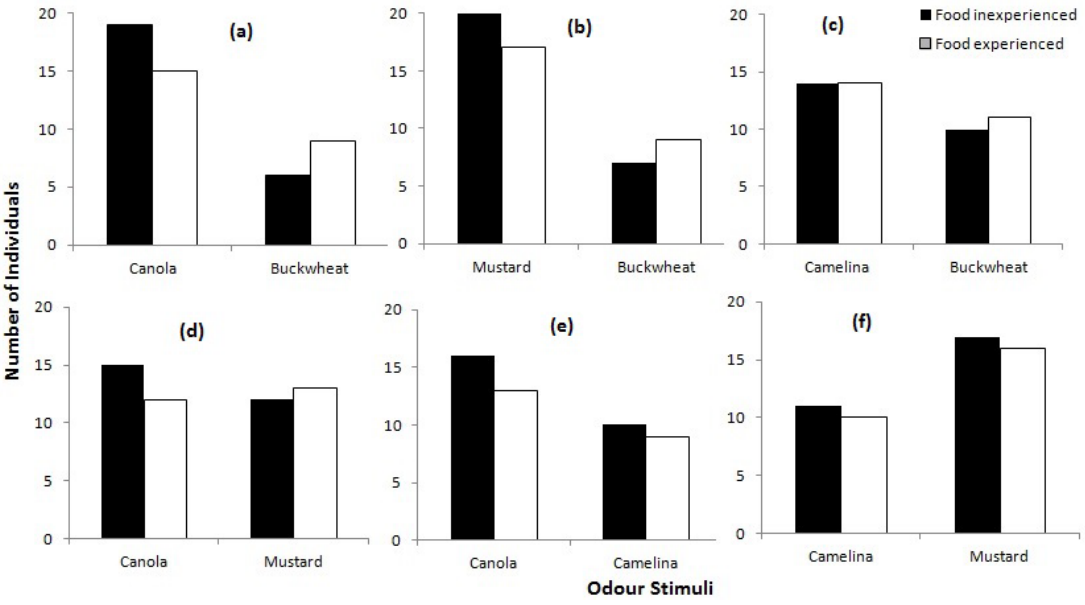

Figure 6: Number of food experienced and food inexperienced *C. cuproviridis* attracted to different odor stimuli offered in a dual choice test (n = 30 for each experiment): a) canola vs. buckwheat; b) canola vs. mustard; c) canola vs. camelina; d) mustard vs. buckwheat; e) camelina vs. mustard; and f) camelina vs. buckwheat.

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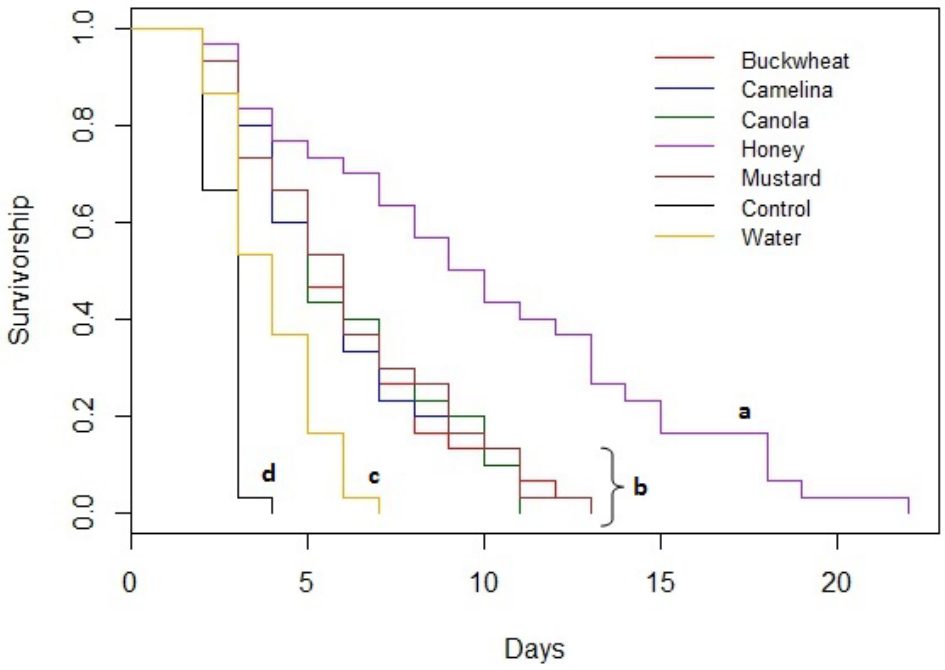

Figure 7: Kaplan–Meier estimates of the survival functions of *C. cuproviridis* when kept with six different treatments (four separate experiments with each of the four plant species, a honey treatment, and a water only treatment) and a no food or water treatment (control). Lower case letters refer to the differences between treatments and the lowercase "b" refers to canola, camelina, mustard and buckwheat (pairwise Cox proportional hazards tests adjusted by sequential Bonferroni for multiple comparisons, $P < 0.05$, n=10 each treatment).

4. **Development of identification tools for parasitoids of cutworms**

This key is being developed as a tool for researchers to identify all sixteen species of parasitoids found attacking cutworms in the Canadian Prairies. The key will be supported with high resolution images of character states to assist identification, and will be published in an online, open-access journal and website. The key will be completed at the end of August 2015.

Part of the delay in this deliverable is due to some taxonomic issues that have arisen with the *Copidosoma* spp.. We have amplified the barcoding gene cytochrome oxidase I for several specimens of *Copidosoma* that have been identified morphologically; *C. bakeri* can be separated from *C. cuproviridis* by the length of the ovipositor, being exerted in the latter and readily visible from the dorsal view. However, when we characterize the species

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using molecular methods, the morphologically identified specimens do not form reciprocal monophyletic groups, suggesting that they may be the same species, or part of a larger species complex (Figure 8).

5. **Additional studies: Assessing the efficacy of entomopathogenic fungi as biocontrol agents of cutworms.**

Natural enemies such as parasitoids and entomopathogenic fungi (EPF) may play an important role by regulating cutworm populations. Parasitized cutworms may feed more and longer than unparasitized cutworms; thus, a high rate of parasitism may exacerbate crop damage and complicate control recommendations. According to our 2012-2014 cutworm data in Manitoba, EPF caused greater mortality to cutworms than parasitoids (Figure 9). Thus, EPF found attacking cutworms may be a more suitable biocontrol agent than parasitic wasps in Manitoba, and possibly in other Prairie provinces.

Entomopathogenic fungi are environmentally friendly pest control agents found in nature. As they are naturally occurring in the soil it is easy to manipulate EPF for biocontrol studies. It has been found that insect death occurs 3 and 5 days after application at optimal conditions. However, few studies have examined the potential for EPF to control cutworms, and thus these species have been underutilized as biological agents for cutworm management. The species of EPF attacking economically important cutworms has not been documented in Manitoba, and in fact, has not been studied for most cutworm species across the country. As cutworms damage crop seedlings, EPF should be applied in the early seedling stage. As many agricultural fields are treated with herbicides prior to sowing or before seedling emergence, herbicides may impact the efficacy of EPF applications in the field. However, there has been limited research on the interactions between herbicides and EPF in field crops. Thus, we will be assessing the efficacy of EPF as biological control agents of cutworms in 2015 and 2016.

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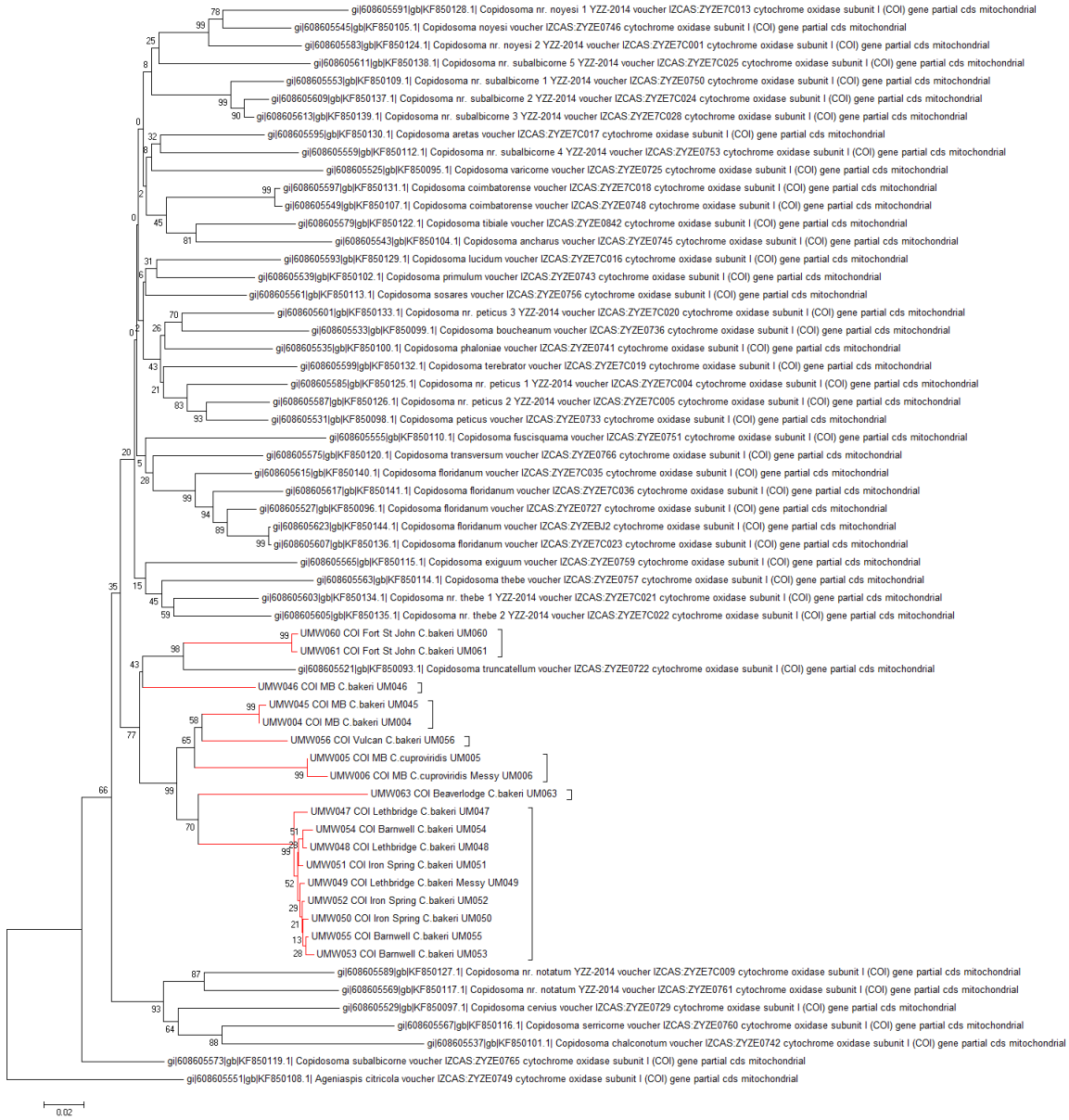

Figure 8. Neighbor-joining tree of *Copidosoma* species under a Kimura-2-parameter model. Specimens collected in this study are highlighted with red branches. Other taxa have been downloaded from the barcode of life database.

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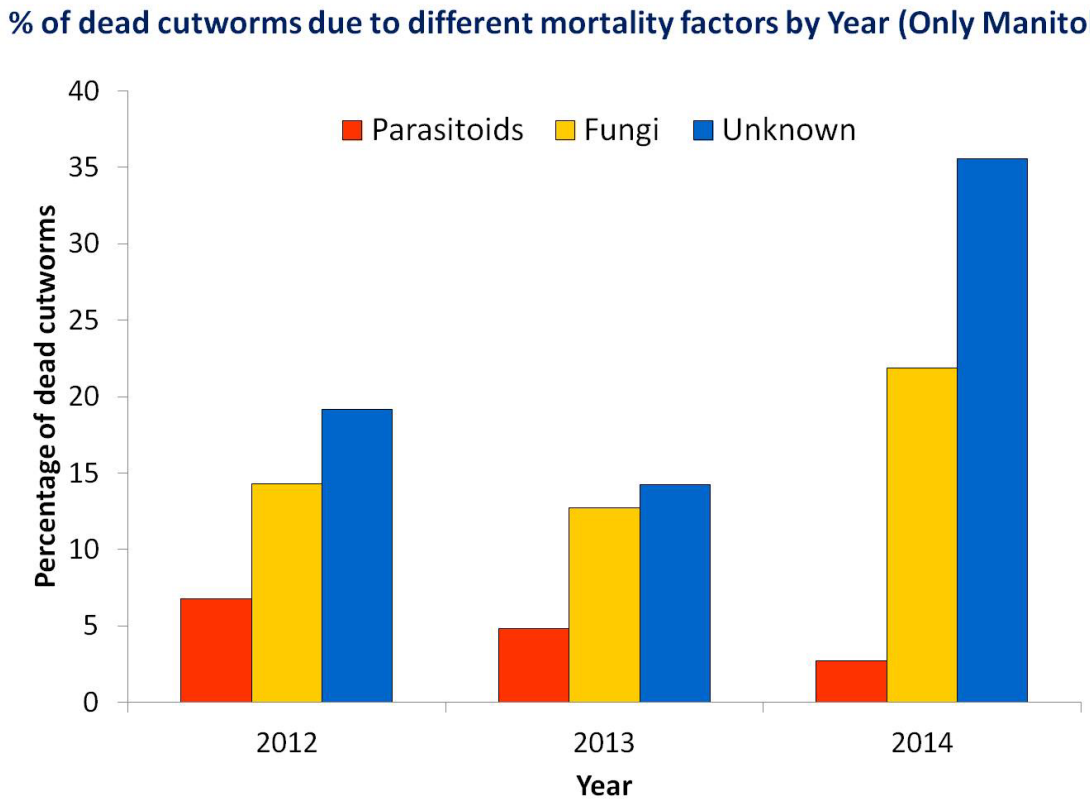

Figure 9. Mortality of cutworms collected in Manitoba from 2012-2014.

Overall Summary:

Generally, multiple species of cutworms cause economic damage to canola across the Canadian prairies every year. Natural enemies (NEs), particularly parasitoids and entomopathogenic fungi (EPF) are natural regulators of cutworms in field crops, but still they are underutilized resources. During our study period, we found that parasitism rates are higher in Alberta compared to Manitoba, but the rate of parasitism is often too low to reduce cutworms below economic levels. The one reason for lower parasitism rates could be, that large acreage monocultures do not provide parasitoids with adequate nutritional resources due to the lack of diversity in the agricultural landscape. In this study, *C. cuproviridis* was the most abundant parasitoid of cutworms identified in Manitoba. It success may be driven by the frequency of canola in the current crop rotations of Manitoba, as the flowering period of canola coincides with the reproductive period of *C. cuproviridis*. Providing alternative food sources may be a strategy to increase the range of parasitoids to control cutworms.

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Reduced crop rotations and elimination of hedgerows and natural edge plantations to maximize cropping space also limits biodiversity. Thus, we have identified and characterized plant species that can attract, maintain, and enhance parasitoids in the community and that show potential for the use in the development of habitat management strategies for cutworm control strategies. During our study we found canola, camelina, mustard and buck wheat are the potential cover crops to enhance parasitoid community in the field. Late planting of cover crops may provide continuous carbohydrate recourses to parasitoids and ensure the flowering period of cover crops coincides with parasitoid emergence. The yellow mixture studied here only flowers for a maximum of 38 days, and thus may be an appropriate mix to provide resources for *Copidosoma*, but may need to be planted late enough to ensure appropriate timing of flowering. It would be interesting to investigate multiple uses of a cover crop such as the yellow mixture studied here. For example, the yellow mixture could be used to provide additional resources for *Copidosoma*, and then be used as a trap crop for fall emerging adult flea beetles, after the parasitoids have left the area.

Further, the most important advantage to the use of NEs to control cutworms is that it typically offers longer term management compared to other chemical control methods. If these strategies can be implemented, it may eliminate the need for chemical control of cutworms, thereby reducing the time and expense involved in their management. However, the low parasitism rates discovered here suggest that they may not be the best target for effective biocontrol of cutworms. Rather, entomopathogenic fungi sprayed early in the growing season may provide much better control of cutworms and this needs to be studied in more depth.

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