

# Alfalfa Insect Survey (2014F)62R

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## Introduction

Alfalfa is an important crop in Alberta, both as forage to provide feed for the province's extensive livestock industry and as seed for export. Despite the importance of alfalfa crops, there are no up-to-date surveys for insects in Alberta alfalfa fields and little data is available regarding the species' range of known pests. Pest management plans and justifications for the registration of plant protection products in alfalfa fields are challenging to create when no up-to-date pest population baselines exist. The lack of knowledge available for alfalfa pest insects leaves the industry vulnerable to new and old risk insect species.

The insect survey underway, in its second year of three, will allow the establishment of baseline population numbers for alfalfa insects, document population range expansion by known pest species, and identify the presence and range of any potential new risk species. The benefits of the insect survey will help inform ongoing and future surveillance work, allowing for informed integrated pest management practices. In addition to helping increase the knowledge of pests in alfalfa fields, a paired study looking at the population dynamic of *Lygus* spp. between alfalfa and canola fields will be performed to determine if populations of *Lygus* spp. feeding on alfalfa move into canola fields following alfalfa harvest.

#### Methods

#### Sweeps

Ninety-nine alfalfa fields in Alberta were selected for the survey: 19 in the Central region, 17 in the Northeast, 15 in the Northwest, 19 in the Peace, and 29 in the South. At each field site a general collection for insects was performed using a sweep net in a 180° motion (see video for <u>demonstration of technique</u>). One hundred sweeps were performed and the collected insects were combined into one bag. The total catch was cleaned in the lab to remove any plant debris and divided into quarters.

A random quarter of each sweep sample was selected and the insects were identified, counted and vialed. Select insects were identified to species (i.e. alfalfa weevil, *Sitona* weevils, Ladybird beetles, lygus bugs, alfalfa plant bugs, minute pirate bugs, twice-stabbed stink bugs, western flower thrip and aphids) while others were identified to family (e.g. alfalfa blotch leafminer) or

to order (Table 1). The results, presented in terms of <sup>1</sup>/<sub>4</sub> of the total sample, were graphed by region and mapped.

Insects	Coleoptera	Hemiptera	Thysanoptera
Order	Other beetles	Other bugs	-
	Beetle larvae	-	
Family	Soft-winged flower beetles	Leafhoppers (Cicadellidae)	Phlaeothripidae
	(Melyridae)	Spittlebug (Cercopoidae)	Aeolothripidae
	Blister beetles (Meloidae)	Other aphids (Aphididae)	
	Ground beetles (Carabidae)	Minute pirate bugs (Anthocoridae)	
	Click beetles (Elateridae)	Damsel bugs (Nabidae)	
Species	Alfalfa weevil	Alfalfa plant bug (Adelphocoris	Western flower
	(Hypera postica)	lineolatus)	thrip
	Sitona (S. lineatus & S.	Lygus bugs (4 species)	(Frankliniella
	cylindricollis)	Pea aphid (Acyrthosiphon pisum)	occidentalis)
	Ladybird beetles (6 species)	Spotted alfalfa aphid ( <i>Therioaphis</i> maculata)	
		Twice-stabbed stink bug	
		(Cosmopepla lintneriana)	

Table 1. Taxonomic levels to which insect samples wer	e identified and tallied.
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Insects	Diptera	Hymenoptera	Orthoptera	Lepidoptera	Neuroptera
Order	Other flies	Wasps	Grasshoppers	Adults	-
	Fly larvae	Sawfly larvae		Caterpillars	
Family	Leaf-miner flies (Agromyzidae) Syrphid flies (Syrphidae)	Bees (Apoidea)	Katydids (Tettigonioidea)	_	Lacewings (Chrysopidae)
Species	-	-	-	-	-

Non-insects	Acari	Collembola	Araneae	Opiliones
Order	Mites	Globular (Symphypleona)	Other spiders	Harvestmen
		Long (Entomobryomorpha)	_	
Family		-	Long jawed	-
			(Tetragnathidae)	
			Orb-weaver (Araneidae)	
			Jumping (Salticidae)	
			Crab (Thomosidae)	
			Running crab	
			(Philodromidae)	
Species		-	-	-

Alfalfa blotch leafminers

At each of the 99 alfalfa fields, where sweeps were performed, alfalfa stems were collected to examine for damage by alfalfa blotch leafminers. Three stems were collected from 10 different

locations in each field for a total of 30 stems. Ten of the 30 stems were selected at random and, for each, three measurements were taken: stem length, the number of leaves, and the number of mines. Only the number of mines was recorded for the remaining 20 stems.

Leaves with mines were cut from the stems and photographed to preserve the appearance of the mine. The mine appearance can be used to identify the species of fly whose larva has been feeding within the leaf. Feeding by the alfalfa blotch miner (*Agromyza frontella* Rondani 1874) creates a mine that thickens as it moves towards the apex of the leaf only to turn back near the mid-rib. The mine of the lesser alfalfa blotch miner (*Agromyza nana* Meigen 1830) is also associated with the mid-rib, but has a shorter, thin tail and a much larger white blotch at the mid-rib. An on-line key was accessed for help using mine characters for the identification of the species likely responsible (<u>http://www.ukflymines.co.uk/Keys/TRIFOLIUM.php</u>). Larvae remaining within the mines will be extracted and be identified using DNA barcoding.

#### Paired Lygus study

Sweeps were performed at eight paired canola and alfalfa fields in the foothills area of Alberta. Each set of paired fields chosen were located across a road or had a common border. The close proximity of the fields will allow accurate documentation of any lygus bug movement between fields before and after alfalfa harvest. The fields were visited once a week for four to five weeks and during each visit two sets of 25 sweeps for canola and alfalfa were performed. The sweep samples were collected into separate bags, taken back to the lab and frozen before identification. The lygus bugs, adults and nymphs, from each sample were counted and identified to species or nymphal stage. Fields were given *a posteriori* designations to match those used by Cárcamo *et al.* (2003): "cut" for field pairs where the alfalfa was harvested early, "check" for pairs where the alfalfa was harvested one to two weeks after the "cut", and "uncut" for pairs where the alfalfa remained unharvested during the collection period.

## **Results and Discussion**

#### Sweeps

The South once again had a larger abundance of pest insects in 2015 than the other four regions (Figure 1). The Central region had the next highest abundance in terms of pest number. The number of predators, i.e. ladybird beetles (adults and larvae), syrphid flies, wasps (parasites and

parasitoids), lacewings (adults and larvae), spiders, harvestmen, minute pirate bugs (adults and nymphs) and damsel bugs (adults and nymphs), was also highest in the South (Figure 1).



Figure 1. Total number of insects averaged per region in 2015

#### Pest insects

#### Alfalfa weevil

Alfalfa weevil (*Hypera postica* Gyllenhal 1813) adults were found mainly in the South and Central regions (Figure 2), with the largest number at one Southern site, Willow Creek Co., reaching 111 in <sup>1</sup>/<sub>4</sub> of the sample alone. The highest numbers of suspect alfalfa weevil larvae were also seen in the South (e.g. 3005 from <sup>1</sup>/<sub>4</sub> of the sample at the same Willow Creek Co. site) (Figure 3). The numbers of both adults and larvae are higher this year than those seen in 2014 and there were also more sites with higher numbers in 2015 than 2014. In 2014, no more than 50 adults were counted from <sup>1</sup>/<sub>4</sub> of the total sample and the larval count did not go over 1,400. In contrast to 2014 numbers, we observed some sites in 2015 to have over 3,000 suspect larvae and over 100 alfalfa weevil adults in <sup>1</sup>/<sub>4</sub> of the total sample.



In 2015, adult alfalfa weevils were found at sites farther north than previously observed, indicating a population expansion into the Northeast and Northwest regions (Figure 2). Larvae were also found at these more northern sites and, using DNA barcoding, they were confirmed to be *Hypera postica* (Figure 3). The alfalfa weevil has not been found at any sites in the Peace, but a larvae of the species *Hypera trivittata* (Say 1831) was found at Greenview County. *Hypera trivittata* has previously been collected in Alberta and while larvae were once collected off of veiny pea (*Lathyrus venosus*), little else is known about its ecology (Titus 1911).

Another weevil species, *Ceutorhynchus punctiger* (Gyllenhal), was often found in fields with high numbers of dandelion in 2014 as the seeds of dandelion are hosts to the larvae. The larvae of this weevil can be confused with that of the alfalfa weevil, leading to potential misidentifications. To decrease the chance of confusion, fields with high numbers of dandelion were avoided in 2015 and will be again in 2016.

# Sitona weevils

The *Sitona* weevils collected at each site were identified to one of two species. Both species, the pea leaf weevil (*S. lineatus* Linnaeus 1758) and the sweet clover weevil (*S. cylindricollis* Fåhraeus 1840), will feed on alfalfa, the adults feeding on the foliage and growing points while the larvae feed on the roots' nitrogen fixing nodules. Feeding by the pea leaf weevil can

potentially cause serious damage in alfalfa fields during the seedling stage, but the sweet clover weevil is not a serious pest of alfalfa and neither species completes their lifecycle on alfalfa plants.

The sweet clover weevil was the most prominent species in alfalfa fields across Alberta (Figure 4). Few sites had pea leaf weevil and never more than one observed from <sup>1</sup>/<sub>4</sub> of the sample. No pea leaf weevil was found in the Northwest or Peace sites (Figure 4).



# Alfalfa plant bug

There were more adult alfalfa plant bugs collected in the South and Peace regions than in the other three regions in 2015 (Figure 5). In all regions there were more nymphs than adults collected, the majority of which were late stage nymphs (Figure 6). The same trend for a higher number of nymphs in sweeps was seen in 2014 and may be the result of the bug's lifecycle. It is the egg stage that overwinters and there are only 1-2 generations per year (High Plains IPM Guide, 2006). Our collection dates likely overlapped with the last few nymphal stages and the newly developed adults.



# Lygus sp.

In each region, the average number of *Lygus* (Hahn) collected was greater in 2015 than in 2014. In 2015, the number of adult lygus bugs was lower than that of nymphal lygus, but was fairly even across all regions (Figure 7). The South and Northeast regions had the largest number of nymphs, reaching a maximum of 415 in 25 sweeps from Cypress County in the South. In every region, the collection in 2015 likely overlapped with the emergence of nymphs from eggs laid by the overwintering adults, which would explain the high number of nymphs of all stages in the sweeps (Figure 8).



The adult lygus specimens were identified to four species: *Lygus lineolaris* (Palisot de Beauvois 1818), *L. keltoni* (Schwartz and Foottit 1998), *L. borealis* (Kelton 1955) and the relatively rare *L. elisus* (Van Duzee 1914) (Figure 9). The tarnished plant bug, *L. lineolaris*, had high numbers in the Northwest, Central and Northeast, but lower numbers in the Peace and South where *L. keltoni* was the most abundant (Figure 10). The species *L. borealis* had relatively low numbers across Alberta while *L. elisus* had even lower numbers and was not found in the Northwest nor Northeast (Figure 10).



#### Aphids, thrips and mites

The average number of pea aphid was highest in the South and relatively even across the rest of Alberta in 2015 (Figure 11). In 2014, the pea aphid was present in higher numbers in the Peace and Northwest and in lower numbers in the other regions. Spotted alfalfa aphid was less numerous than pea aphid in both years, but it was found in every region (Figure 12). The black potato aphid was found only rarely and always in small numbers.



Thrip numbers were once again highest in the South and Central regions of Alberta in 2015 (Figure 11) as they were in 2014; however, the average numbers from each region in 2015, with the exception of the Peace, were double those seen in 2014. Thrips were caught at every site and on average in high numbers with the exception of the Peace region where only one site had over 100 thrips in <sup>1</sup>/<sub>4</sub> of the sample (Figure 13). The western flower thrip made up the majority of the thrip count, with only a few other species (e.g. the red clover thrip, Phlaeothripidae) collected. Species within the Aeolothripidae family are often predatory, but only a few were collected across all regions.

The mites were not identified to predatory or pest species due to the difficulty in mite identification. The number of mites was highest in the Northeast, Central and South regions (Figure 11), all with the highest count being over 300 in <sup>1</sup>/<sub>4</sub> of the total smaple (Figure 14).



Spittlebugs and leafhoppers

Few spittlebugs were found in 2015 compared to 2014, potentially due to the dry weather. Leafhopper numbers in 2015 remained steady across all regions (Figure 15) and were similar to those seen last year. In 2014 and 2015, the number of adult leafhoppers was far greater than the number of nymphs; however, the number of spittlebug nymphs in 2015 was larger than the number of adults (Figure 16), which contrasts the trend seen in 2014 for more adult spittlebugs.



Some of the leafhopper and spittlebug specimens caught, both adults and nymphs, were observed to have a sac(s) protruding from their bodies (Figure 17a). These sacs were able to be explained as developing parasitoid larva when two adult females of the genus *Gonatopus* (Ljungh 1810) were found in sweeps from two sites. Females of *Gonatopus* are wingless parasitoids that resemble ants. They have modified forelegs (Figure 17b) that allow them to grasp onto a host, either a spittlebug or leafhopper, and lay an egg. Though these parasitoids are not available commercially, the presence of adult females and widespread appearance of parasitoid sacs indicates them as an established natural biocontrol (Figure 17c).



#### Grasshoppers and katydids

Though some grasshopper species are more destructive than others, alfalfa is not a preferred host for many species (Mulkern, *et al.* 1962), which means they are often seen only in low numbers in alfalfa fields. Few adult grasshoppers were caught as the survey was likely conducted prior to adult development, but at some sites there were high numbers of nymphs. Grasshopper nymphs are difficult to identify to species and were, therefore, not further taxonomically divided. The largest numbers of nymphs in 2015 (e.g. 2487 from ¼ of the sample at a single site) were observed in the Peace region (Figure 18), a region that had under 10 grasshoppers at every site in 2014. Unlike 2014, there were few sites in 2015 with no grasshoppers and the numbers caught in each sample were higher. No region had high numbers of katydids in the 2015 alfalfa sweeps, but they were observed in all regions (Figure 19).



# Lepidoptera

Adult Lepidoptera were rarely caught and few of the adults that were caught (Figure 20) were in a condition to allow ready identification, beyond moth or butterfly. Small white moths and European skippers accounted for the majority of the adult Lepidoptera caught. More caterpillars were collected than adults, but, with the exception of the European skipper, they too were not identified to species (Figure 20). The sites with larger numbers of caterpillars in 2015 sweeps were in the same areas as those in 2014.



## Predators

All regions across Alberta had a similar breakdown in predator proportion (Figure 21). Wasps, spiders, harvestmen and minute pirate bugs were the most prominent predator in terms of numbers (Figure 21). However, some predators (e.g. lacewing and syrphid fly larvae) are more voracious than others and may have more impact on the pest population than their numbers imply.



Figure 21.

# Ladybird beetles

Ladybird beetles were found in highest numbers in the South in 2015 (Figure 22) as they were in 2014. The larvae of ladybird beetles, like the adults, are effective predators and, therefore, ladybird beetles are favoured as biocontrol agents for pests. The popularity of ladybird beetles as predators has led to the introduction of non-native species to North America. These invasive species are believed to cause the decline and displacement of native species (Snyder *et. al.* 2004). Seven species of ladybird beetles were collected from the 2015 alfalfa sweeps. Three species collected in the 2014 alfalfa sweeps, thirteen spot, wee-tiny and five spot, were not found in 2015, but an additional species, the convergent lady beetle, was observed (Figure 23).



The seven spotted lady beetle, the most prominent species observed in 2015, is an alien species to Canada; however, the parenthesis lady beetle and the three-banded lady beetle, the next most prominent species, are both native. Native species outnumbered the seven spotted lady beetle in the Peace region (Figure 23). The South was the only region in which the convergent, sinuate and expergate lady beetle species were observed (Figure 23).

# Wasps

Wasps can be pollinators as well as predators and parasitoids. All the wasps were grouped together, but the majority of wasps collected were small parasitoids from families such as Ichneumonidae, in which the genus *Bathyplectes* that includes parasitoids of the alfalfa weevil is found, and Braconidae. A few sites in the Northeast and South had high numbers of wasps (Figure 24), but the majority of sites had between 1 and 25 wasps (Figure 25).



## Spiders and Opiliones

Spiders are obligate predators as are many species of opiliones, commonly known as daddy long legs or harvestmen. Both spiders and harvestmen were found at sites in all regions (Figure 26) and at higher numbers than in 2014. In 2015, the Peace region had the lowest numbers of both spiders and harvestmen (Figure 27). Harvestmen and some families of spiders (e.g. wolf spiders and jumping spiders) must hunt prey, while members of other spider families are sit and wait predators using either webs (e.g. orb-weavers) or ambush (e.g. crab spiders).

Figure 26.



The spider family Tetragnathidae or Long jawed spiders was the most common family found, except in the Peace region where crab spiders (Thomisidae) and orb-weavers (Araneidae) dominated (Figure 28). Few jumping spiders (Salticidae) and wolf spiders (Lycosidae) were found, likely due to their speed and nomadic lifestyle (Figure 28).



Lacewings, damsel bugs and minute pirate bugs

As in 2014, there were few lacewings, both adult and larval, and few damsel bugs collected in 2015 (Figure 29). The South had the largest average number of both lacewings and damsel bugs (Figure 29). Damsel bugs will prey on pest species as both nymphs and adults whereas the larva of lacewings is the main predacious stage.



Higher numbers of minute pirate bugs were seen at most sites than lacewings and damsel bugs (Figure 30). Like damsel bugs, minute pirate bugs feed on pest species in both the nymphal and adult stage. Their numbers were significantly higher in the South in 2015 than was observed in 2014, as well the Central and Northeast regions had a higher population in 2015 (Figure 29).

# Syrphid flies

Syrphid flies were observed in low numbers in the 2015 as they were in 2014 (Figure 31). The larvae of syrphid flies can be voracious predators on their primary prey aphids, but are impossible to distinguish morphologically from other fly larvae.



## Alfalfa blotch leafminers

The average number of suspect Agromizidae leaf mines was lower in every region except the South in 2015 (Figure 32) compared to 2014. Where the Northwest and Peace averaged more than 1 mine per stem in 2014, no region averaged over 0.27 mines per stem in 2015 (Figure 32). In 2015, select sites in the Peace had high numbers of mines on 30 stems, but few suspect Agromizidae adults were found (Figure 33). More suspect adults were observed in other regions, but at low numbers overall (Figure 33).



Using the on-line key for tentative identification, the mines were visually assigned to either *A*. *frontella* (Figure 34a) or *A. nana* (Figure 34b). *Agromyza nana* damage was more prevalent in the Peace, South and Northeast regions (Figure 34c), all areas where more leafminer damage was observed (Figure 33). Confirmation of larva and adult identification to species will be made using DNA barcodes, but is not available now.



There is a positive relationship between the number of mines on 10 stems and the average length of 10 stems (cm) as well as the number of mines and the number of leaves on 10 stems, but no significant correlation. This positive but non-significant correlation was observed in 2014 as well.

## Paired Lygus study

Exhaustive analyses, including t-Tests, ANOVA and Kruskal-Wallis tests have yet to be run on the data collected for the paired *Lygus* study, but the trends in species composition are presented in Table 2. The species composition, based on percentage, in four canola fields (Balzac, NRV, MVSouth and N site) appears to be unaffected by alfalfa harvest (Table 2). This trend appears to be untrue for the other four sites (MVNorth, MV/RD, RD and Penhold); however, observation of the population dynamics within canola fields may explain the changes in species composition.

The increase in *L. lineolaris* following alfalfa harvest at the "cut" MCNorth site (Figure 35a) corresponds to population increases of this species at "check" sites (e.g. MC/RD) before alfalfa harvest (Figure 35b). Similarly, the percentage of *L. borealis*, a species that is seldom dominate in canola fields (Cárcamo *et al.* 2003), increased at three "check" sites following alfalfa harvest (Table 2). As *L. borealis* is more common in alfalfa fields, these peaks (e.g. Figure 35b) may be attributed to the alfalfa harvest, but they actually correspond to a population increase seen at a "cut" site (Figure 35a) well after alfalfa harvest. Therefore, the changes in species composition and number in canola fields can be attributed to within field population dynamics. The

commonly held belief that lygus bugs move from alfalfa to canola following harvest is likely due to alfalfa harvest preceding natural population booms of different *Lygus* species in canola fields.







Table 2. Total number and percent of total (in brackets) of the *Lygus* spp. collected from each crop type at every paired field site, both before and after alfalfa crops were harvested. Highlighted entries indicate increases, following alfalfa harvest, in the proportion of a species in canola fields that was more prevalent in alfalfa fields prior to harvest.

		Cut				Check							Uncut				
Balzac		zac	MVNorth		NRV		MV/RD		RD		Penhold		MVSouth		N site		
Species	Timing	Alf.	Can.	Alf.	Can.	Alf.	Can.	Alf.	Can.	Alf.	Can.	Alf.	Can.	Alf.	Can.	Alf.	Can.
<i>L</i> .	Before	1	9	<mark>50</mark>	<mark>48</mark>	8	26	67	263	75	147	89	42	1	31	62	72
lineolaris		(11.1)	(11.8)	<mark>(43.5)</mark>	<mark>(31.4)</mark>	(17.4)	(14.4)	(30.9)	(64.6)	(35)	(64.8)	(41.6)	(50)	(1.9)	(16.6)	(28.7)	(51.4)
	After	-	38	-	<mark>207</mark>	-	21	-	158	-	48	-	44	-	6	-	-
			(19.5)		<mark>(67.4)</mark>		(14.2)		(67.5		(47.1)		(36.7)		(5)		
<i>L</i> .	Before	5	40	37	66	23	93	<mark>83</mark>	<mark>26</mark>	<mark>60</mark>	<mark>26</mark>	<mark>77</mark>	<mark>19</mark>	40	95	109	32
borealis		(55.6)	(52.6)	(32.2)	(43.1)	(50)	(51.4)	<mark>(38.2)</mark>	<mark>(6.4)</mark>	<mark>(28)</mark>	<mark>(11.5)</mark>	<mark>(36)</mark>	<mark>(22.6)</mark>	(74.1)	(50.8)	(50.5)	(22.9)
	After	-	97	-	64	-	77	-	<mark>55</mark>	-	<mark>28</mark>	-	<mark>49</mark>	-	62	-	-
			(49.7)		(20.8)		(52)		<mark>(23.5)</mark>		<mark>(27.5)</mark>		<mark>(40.8)</mark>		(52.1)		
L. keltoni	Before	3	27	28	39	15	62	67	116	78	51	48	23	13	61	216	36
		(33.3)	(35.5)	(24.3)	(25.5)	(32.6)	(34.3)	(30.9)	(28.5)	(36.4)	(22.5)	(22.4)	(27.4)	(24.1)	(32.6)	(20.8)	(25.7)
	After	-	60	-	36	-	50	-	21	-	26	-	27	-	51	-	-
			(30.8)		(11.7)		(33.8)		(8.97)		(25.5)		(22.5)		(42.9)		

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