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Seasonal abundance of key alfalfa (Fabaceae) seed production pests and their natural enemies in southern Alberta, Canada

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Abstract

The economic importance of insect pests in agricultural fields and the potential biological control by their natural enemies warrant foundational studies for the development of integrated pest management strategies. An insect survey was conducted in alfalfa (Fabaceae) seed production fields in southern Alberta, Canada, during the bud, flowering, and seed crop stages in 2020 and 2021. We examined the seasonal abundance of *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), *Lygus* spp. Hahn (Hemiptera: Miridae), and 12 natural enemy taxa. We also examined the seasonal abundance, richness, and diversity of generalist predators and the seasonality of the two *H. postica* parasitoids, *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) and *Oomyzus incertus* (Ratzeburg) (Hymenoptera: Eulophidae). The seasonality of pests and natural enemies was inconsistent between years. *Hypera postica* larvae, *B. curculionis*, and *O. incertus* were present from mid-June to mid-August. However, we detected no correlations between *H. postica* larvae and its two parasitoids in either year. A number of generalist predators were correlated with *A. lineolatus* and *Lygus* spp. Further research is needed to understand the effects of environmental and biotic factors on the seasonality of pests and natural enemies in alfalfa seed production fields, and the insects' trophic interactions.

Introduction

Biological control of pests by their natural enemies represents a valuable regulating ecosystem service (Symondson *et al.* 2002; Losey and Vaughan 2006). The economic importance of insect pests in agricultural fields and the potential biological control by their natural enemies warrant foundational studies for the development of conservation and integrated pest management strategies (Tscharrntke *et al.* 2007). This has become more pressing with global insect population declines (Hallmann *et al.* 2017; Leather 2018; Goulson 2019; Wagner *et al.* 2021) and the potential, exacerbating effects of climate change on pest issues in agricultural fields (Cannon 1998; Estay *et al.* 2009; Tonngang *et al.* 2022). Alfalfa, *Medicago sativa* Linnaeus (Fabaceae), is one of the most important forage crops, particularly in temperate regions of the world, and is often grown for animal forage and for its nitrogen-fixing abilities (Burity *et al.* 1989; Soroka and Otani 2011;

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Edde 2021). In Canada, alfalfa dominates forage seed production with over 3 million hectares grown annually, of which 73% is produced in the Canadian Prairie Provinces of Alberta, Saskatchewan, and Manitoba (Statistics Canada 2022).

A variety of insect pests occur in alfalfa fields in the Canadian prairies, including the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), the pea aphid, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae), the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) (Hemiptera: Aphididae), lygus bugs, *Lygus* spp. Hahn (Hemiptera: Miridae), the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), the alfalfa seed chalcid, *Bruchophagus roddi* Gussakovsky (Hymenoptera: Eurytomidae), and the alfalfa blotch leafminer, *Agromyza frontella* (Rondani) (Diptera: Agromyzidae) (Hobbs *et al.* 1959; Harper 1988; Schaber and Entz 1988; Uddin 2005; Sim and Meers 2017). In addition, Sim and Meers (2017) identify minor pests in forage alfalfa, including a three-species complex of *Sitona* spp. German weevils (Coleoptera: Curculionidae), thrips (Thysanoptera), and the lucerne flea, *Sminthurus viridis* (Linnaeus) (Symphypleona: Sminthuridae), some of whose populations occasionally can cause significant damage.

Hypera postica is considered one of the most economically important pests of alfalfa in North America (Soroka *et al.* 2020). It was introduced from Eurasia in the early 1900s (Titus 1911) and was first reported in southern Alberta in 1954 (Hobbs *et al.* 1959). Since then, *H. postica* has undergone a rapid geographical expansion in the Canadian prairies in the past two decades (Soroka *et al.* 2020). *Hypera postica* is univoltine in Canada and the northern United States of America (Bereza 1970). Overwintering adults emerge in spring and lay several clusters of approximately 10 eggs on plant stems (Whitford and Quisenberry 1990). Eggs develop over two weeks, larvae feed and mature over a period of 3–5 weeks, and adults continue to feed briefly before hibernation (Soroka *et al.* 2020). During both the larval and adult stages, the insect feeds on alfalfa foliage, but larvae peak during the early- and mid-bloom stages and are particularly injurious to the crop during this period (Soroka and Otani 2011; Soroka *et al.* 2020). Management of *H. postica* on the Canadian prairies includes the use of monitoring, economic thresholds, insecticide applications, and biological control. Previous studies have investigated biological control of *H. postica* in alfalfa fields in the Canadian prairies by two introduced parasitoids, *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) and *Oomyzus incertus* (Ratzeburg) (Hymenoptera: Eulophidae) (Soroka 2013; Soroka *et al.* 2020). Nonetheless, the expansion of *H. postica* is relatively recent, and parasitoid population buildup can take several years (Soroka *et al.* 2020), warranting monitoring and surveying.

Several generalist predators have been documented in alfalfa fields in the Canadian prairies, including the following: ground beetles (Coleoptera: Carabidae); damsel bugs (Hemiptera: Nabidae); big-eyed bugs (Hemiptera: Geocoridae); lacewings (Neuroptera: Chrysopidae); lady beetles (Coleoptera: Coccinellidae); hoverfly larvae (Diptera: Syrphidae); minute pirate bugs, *Orius insidiosus* (Say) (Hemiptera: Anthracoridae); *Aeolothrips fasciatus* (Linnaeus) (Thysanoptera: Aeolothripidae); harvestmen (Opiliones: Phalangidae); and numerous spider families (Araneae) (Harper 1988; Uddin 2005; Sim and Meers 2017). Nonetheless, the seasonality and spatiotemporal relationships between *H. postica* and the generalist predators occurring in alfalfa fields in the Canadian prairies have been scarcely studied (but see Schaber and Entz 1988 and Uddin 2005).

The variety of insect pests and natural enemies found in alfalfa agroecosystems and the importance of this crop in the Canadian prairies warrant more research into the seasonality of these taxa for the development of integrated pest management strategies. In the present study, we investigated the seasonal abundance of three major pests in alfalfa seed production fields in southern Alberta, Canada: *H. postica*, *A. lineolatus*, and *Lygus* spp. We also examined the seasonal abundance, richness, and diversity of generalist predators and the seasonality of two *H. postica* parasitoids, *B. curculionis* and *O. incertus*. Finally, we examined correlations between pests and natural enemies and discuss their potential under a biological control framework.

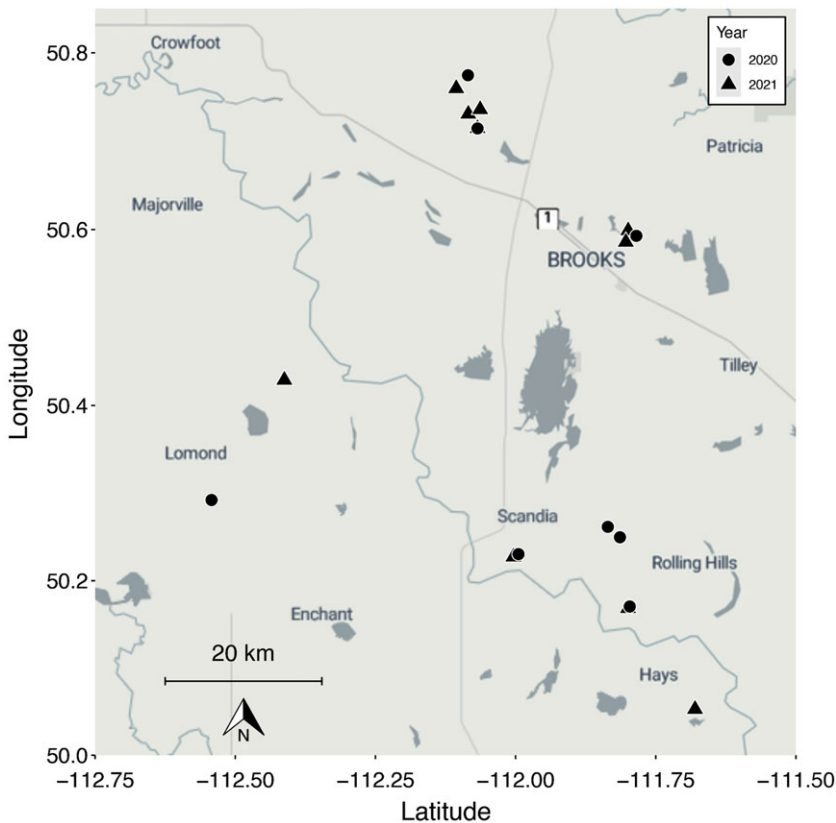


Figure 1. Locations of alfalfa seed production fields surveyed in 2020 and 2021 in southern Alberta. The thick, winding grey line represents the Bow River.

Materials and methods

Field sites and insect sampling

An insect survey targeting a subset of pests and natural enemies was conducted in alfalfa fields in southern Alberta. Sixteen irrigated alfalfa seed production fields in their second or third year of production were sampled during two years (2020: $n = 8$; 2021: $n = 10$; two fields were sampled in both years; Fig. 1). Sampled fields were quarter sections (approximately 0.65 km^2) separated by a minimum distance of 1.5 km (10.94 ± 11.92 , mean \pm standard error) and were managed by growers according to standard agronomic practices, including insecticide application when necessary. Insecticide application data were requested, but only a partial dataset was obtained; therefore, it is not included here.

Fields were monitored weekly and sampled at three crop stages, based on Mueller and Teuber (2008): bud (stage 4), start of flowering (stage 6), and full seed (stage 8; Table 1). Insects within the crop canopy were sampled using a standard sweep net (38.1 cm diameter) along a 100-sweep transect. The sweeping transect began 10 m into the field and consisted of 25 sweeps in a 45° angle perpendicular to the field edge, followed by 50 sweeps parallel to the field edge and 25 more sweeps in a 45° angle opposite to the first. Sweep net samples were collected by the same individual within each year for consistency. Fields were sampled between 08:00 and 18:45 hours, local time, when temperatures were above 15°C . Samples were collected into resealable plastic bags and transported inside an insulated container with ice to the laboratory, where they were stored at -20°C before identification.

Table 1. Abundance of generalist predators and two *H. postica* parasitoids collected in alfalfa seed production fields in southern Alberta, 2020 and 2021. Values indicate means of individuals per 100 sweeps. Eight and 10 fields were sampled in 2020 and 2021, respectively. Collection dates in 2020 occurred between 10 and 12 June, between 4 and 6 July, and between 10 and 11 August during the bud, flower, and seed stages, respectively. Collection dates in 2021 occurred on 16 June, 21 July, and 11 August during the bud, flower, and seed stages, respectively

Crop stage	Order	Family	Genus/species	2020		2021	
				Mean	Standard error	Mean	Standard error
Bud	Araneae ^{† §}			21.5	15.54	9	2.01
	Coleoptera	Coccinellidae	<i>Coccinella septempunctata</i> [§]	23.38	2.34	6	2.45
			<i>Hippodamia convergens</i> [‡]	0.5	0.27	0.1	0.1
			<i>Hippodamia parenthesis</i> [‡]	1.25	0.53	–	–
	Hemiptera	Anthocoridae	<i>Orius insidiosus</i> ^{§ ¶}	2.25	0.75	89.4	19.83
		Nabidae	<i>Nabis</i> spp. ^{‡ §}	12.75	1.25	2.4	0.67
	Hymenoptera	Eulophidae	<i>Oomyzus incertus</i> [¶]	4.38	2.00	1.5	1.39
		Ichneumonidae	<i>Bathyplectes curculionis</i> ^{† ‡ ¶}	9.34	2.56	1.2	0.47
	Neuroptera	Chrysopidae	<i>Chrysoperla carnea</i> ^{‡ §}	0.75	0.41	1.2	0.51
Flower	Araneae ^{† §}			13	4.86	22	6.18
	Coleoptera	Coccinellidae	<i>Coccinella septempunctata</i> [§]	12.75	6.53	5.9	1.05
			<i>Coleomegilla maculata</i>	–	–	0.1	0.1
			<i>Hippodamia parenthesis</i> [‡]	0.5	0.27	1.00	0.37
			<i>Hippodamia convergens</i> [‡]	–	–	1.3	0.5
			<i>Hippodamia tredecimpunctata</i> ^{‡ §}	–	–	0.1	0.1
			Lady beetle larvae [*]	–	–	8.1	3.51
	Hemiptera	Anthocoridae	<i>Orius insidiosus</i> ^{§ ¶}	4.75	1.81	199.6	50.1
		Nabidae	<i>Nabis</i> spp. ^{‡ §}	2.89	1.20	12.6	5.69
	Hymenoptera	Eulophidae	<i>Oomyzus incertus</i> [¶]	–	–	19.1	9
Ichneumonidae		<i>Bathyplectes curculionis</i> ^{† ‡ ¶}	3.25	0.59	3.4	1.1	

(Continued)

Table 1. (Continued)

Crop stage	Order	Family	Genus/species	2020		2021	
				Mean	Standard error	Mean	Standard error
Seed	Neuroptera	Chrysopidae	<i>Chrysoperla carnea</i> ^{‡ §}	1	0.63	22.9	8.22
	Araneae ^{† §}			12.38	2.56	13.5	4.03
	Coleoptera	Coccinellidae	<i>Coccinella septempunctata</i> [§]	9.25	2.36	11.7	9.73
			<i>Coccinella transversoguttata</i> [‡]	–	–	0.3	0.21
			<i>Hippodamia parenthesis</i> [‡]	1.00	0.42	1	0.7
			<i>Hippodamia convergens</i> [‡]	–	–	3.9	1.99
			Lady beetle larvae [†]	54.38	34.26	32.8	20.24
	Hemiptera	Anthocoridae	<i>Orius insidiosus</i> ^{§ ¶}	52.63	13.16	155.9	26.12
		Nabidae	<i>Nabis</i> spp. ^{‡ §}	11.5	3.51	28.6	6.88
	Hymenoptera	Eulophidae	<i>Oomyzus incertus</i> [¶]	1	0.46	0.5	0.31
Ichneumonidae		<i>Bathyplectes curculionis</i> ^{‡ † ¶}	0.13	0.13	0.6	0.27	
Neuroptera	Chrysopidae	<i>Chrysoperla carnea</i> ^{‡ §}	8.5	2.47	20.5	6.54	

*Individuals were not identified to genus or species and were not used in the species richness assessments.

†Taxon reported by Hobbs *et al.* (1959).

‡Taxon reported by Harper (1988).

§Taxon reported by Uddin (2005).

||Taxon reported by Sim and Meers (2017).

¶Taxon reported by Soroka *et al.* (2020).

Sample identification

Collected arthropods were classified as pests or natural enemies based on Harper (1988) and Soroka and Otani (2011) and were identified to species or genus using taxonomic keys (Marshall 2006; Larson 2013; Soroka *et al.* 2020) and reference specimens provided by Julie Soroka (Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada). Due to the high volume of specimens collected and to time constraints, we limited our observations to three key pests in alfalfa, namely *H. postica*, *A. lineolatus*, and *Lygus* spp., as well as to their potential generalist predators and to two *H. postica* parasitoids, *B. curculionis* and *O. incertus* (Table 1). Parasitoids related to other pests were present in our samples but were not investigated. Voucher specimens of identified taxa were deposited in the Strickland Museum of Entomology, University of Alberta, Edmonton, Alberta, Canada (UASM417149 to UASM417182).

Data analysis

All analyses were conducted in R, version 4.2.1 (R Core Team 2022). Seasonal abundance was examined across three crop stages for *H. postica*, *Lygus* spp., *A. lineolatus*, *B. curculionis*, and *O. incertus*. Using the Stats (R Core Team 2018) and PMCMR (Pohlert 2021) packages, we computed Friedman tests, then Conover multiple comparisons (*P*-values adjusted using the Benjamini–Hochberg method), because data were neither normally distributed nor independent across time points (Zar 2010).

To investigate seasonal richness and diversity of generalist predators across crop stages, Hill numbers were used for richness ($q = 0$) and Simpson diversity ($q = 2$) using the package iNEXT (Hsieh *et al.* 2016). Richness and diversity were calculated at the genus level after sample size-based rarefaction in iNEXT, for which the number of individuals per sample were standardised to $2m$, where m is the lowest sample size found per year (Chao *et al.* 2014; Hsieh *et al.* 2016). Specimens not identified to genus or species (Table 1) were not included in the richness and diversity analyses. *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) was excluded from the analysis because only one individual was caught during the two years of sampling. Richness and diversity of generalist predators were compared between crop stages using Friedman tests, followed by Conover multiple comparison tests (*P*-values adjusted using the Benjamini–Hochberg method).

Abundance correlations between pests and potential natural enemies were examined using repeated measured correlations with the rmcrr package (Bakdash and Marusich 2017) to account for non-independence between time observations. The *P*-values were adjusted using the Benjamini–Hochberg method.

Results

Seasonal abundance of *Lygus* spp. and *A. lineolatus*

Lygus spp. showed inconsistent abundance trends across crop stages between years. *Lygus* spp. abundance did not differ between crop stages in 2020 ($\chi^2 = 5.25$, $df = 2$, $P > 0.05$) but did in 2021 ($\chi^2 = 6.2$, $df = 2$, $P = 0.045$). In 2021, *Lygus* spp. abundance was highest during the seed stage (351.4 ± 75.11 (standard error) individuals per 100 sweeps) compared to the bud (96.9 ± 36.74 (standard error) individuals per 100 sweeps) and the flower (175.6 ± 50.6 (standard error) individuals per 100 sweeps) stages and did not differ between the bud and flower stages (Fig. 2).

Adelphocoris lineolatus abundance differed between crop stages in 2020 ($\chi^2 = 9.75$, $df = 2$, $P = 0.008$) and 2021 ($\chi^2 = 6$, $df = 2$, $P = 0.049$). In 2020, *A. lineolatus* abundance was highest during the bud (92.88 ± 46.13 (standard error) individuals per 100 sweeps) and seed (43 ± 16 (standard error) individuals per 100 sweeps) stages compared to the flower stage (5.25 ± 1.46 (standard error) individuals per 100 sweeps) and did not differ between the bud and seed stages. In

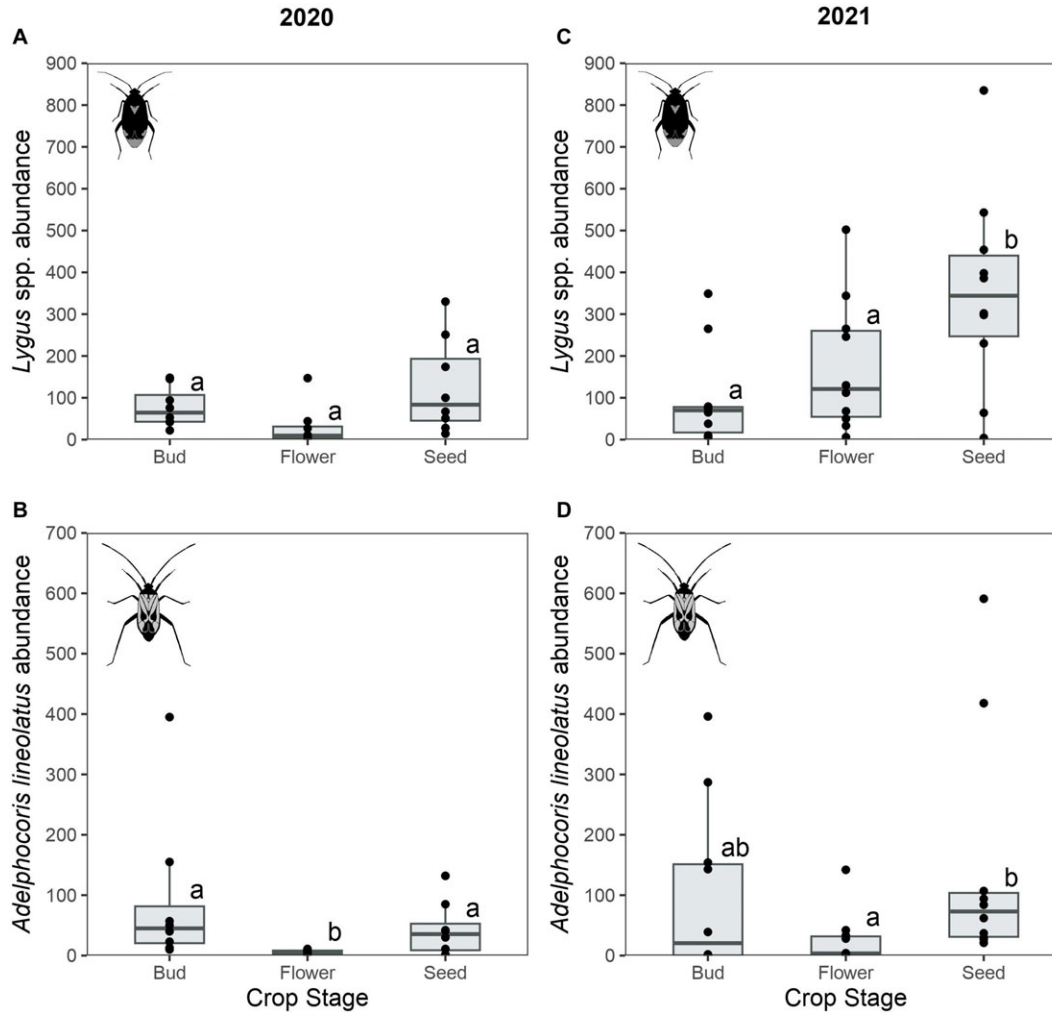


Figure 2. *Lygus* spp. and *Adelphocoris lineolatus* abundances, sampled at three alfalfa crop stages in 2020 and 2021. Values indicate individuals collected per 100 sweeps and include immature and adult insects: **A**, *Lygus* spp. abundance in 2020; **B**, *A. lineolatus* abundance in 2020; **C**, *Lygus* spp. abundance in 2021; and **D**, *A. lineolatus* abundance in 2021. Letters indicate statistical differences between values at each crop stage, based on Conover tests, with *P*-values adjusted with the Benjamini–Hochberg method.

2021, *A. lineolatus* abundance was higher during the seed (147 ± 61.69 (standard error) individuals per 100 sweeps) and bud (102.2 ± 44.77 (standard error) individuals per 100 sweeps) stages compared to the flower stage (25.7 ± 13.84 (standard error) individuals per 100 sweeps; Fig. 2).

Predator seasonal abundance, richness, and diversity

In 2020, there were 40.88 ± 2.48 (standard error), 21.88 ± 8.30 (standard error), and 82.88 ± 13.32 (standard error) predators identified to genus or species per 100 sweeps at the bud, flower, and seed stages, respectively (Table 1). In 2021, there were 99.1 ± 21.42 (standard error), 243.4 ± 55.42 (standard error), and 221.9 ± 35.29 (standard error) predators identified to genus or species per 100 sweeps at the bud, flower, and seed stages, respectively (Table 1). Rarefied richness and diversity of predators showed inconsistent trends across years. No differences were observed in predator richness ($\chi^2 = 0.75$, $df = 2$, $P > 0.05$) or diversity ($\chi^2 = 0.25$, $df = 2$, $P > 0.05$) between crop stages in 2020 (Fig. 3). In 2021, however, significant differences were identified in predator richness ($\chi^2 = 6.2$, $df = 2$, $P = 0.045$) and diversity ($\chi^2 = 8$, $df = 2$, $P = 0.018$) across crop stages. Predator richness in 2021 was higher during the seed stage (3.14 ± 0.17 standard error) compared to the bud stage (2.36 ± 0.17 standard error) but did not differ between the bud and flower stages (3.27 ± 0.31 standard error) or between the flower and seed stages (Fig. 3). Similarly, predator diversity in 2021 was higher during the seed stage (1.70 ± 0.09 standard error) compared to the bud stage (1.25 ± 0.06 standard error) but did not differ between the bud and flower stages (1.79 ± 0.28 standard error) or the flower and seed stages (Fig. 3).

Seasonal abundance of *H. postica* larvae, *B. curculionis*, and *O. incertus*

Hypera postica larval abundance trends differed between crop stages inconsistently across 2020 ($\chi^2 = 13$, $df = 2$, $P = 0.002$) and 2021 ($\chi^2 = 14.6$, $df = 2$, $P < 0.001$). In 2020, *H. postica* larval abundance was higher during the flower stage (1703.63 ± 658.78 (standard error) individuals per 100 sweeps) compared to the bud (433.38 ± 128.45 (standard error) individuals per 100 sweeps) and seed (26.13 ± 9.65 (standard error) individuals per 100 sweeps) stages and was higher during the bud stage compared to the seed stage (Fig. 4). In 2021, *H. postica* larval abundance was highest during the bud stage (492.7 ± 143.01 (standard error) individuals per 100 sweeps) compared to flower (81.6 ± 26.95 (standard error) individuals per 100 sweeps) and seed (9.9 ± 2.43 (standard error) individuals per 100 sweeps) stages and was higher during the flower stage compared to the seed stage (Fig. 4). Over the two years of this study, the two *H. postica* parasitoids were found in alfalfa fields at low but varying numbers between crop stages across years.

The abundance of *B. curculionis* varied significantly across crop stages in 2020 ($\chi^2 = 13$, $df = 2$, $P = 0.002$) but not in 2021 ($\chi^2 = 4.06$, $df = 2$, $P = 0.13$). In 2020, *B. curculionis* abundance decreased with crop stage and was highest during the bud stage (9.34 ± 2.56 (standard error) individuals per 100 sweeps) compared to the flower (3.25 ± 0.59 (standard error) individuals per 100 sweeps) and the seed (0.125 ± 0.125 (standard error) individuals per 100 sweeps) stages, differing also between flower and seed stages (Fig. 4).

The abundance of *O. incertus* varied between crop stages in 2021 ($\chi^2 = 10.89$, $df = 2$, $P = 0.004$) but not in 2020 ($\chi^2 = 4.88$, $df = 2$, $P > 0.05$). It should be noted that in 2020, no *O. incertus* individuals were collected during the flower stage. In 2021, *O. incertus* abundance was higher at the flower stage (19.1 ± 9 standard error) individuals per 100 sweeps) compared to the bud (1.5 ± 1.39 (standard error) individuals per 100 sweeps) and seed (0.5 ± 0.31 (standard error) individuals per 100 sweeps) stages (Fig. 4).

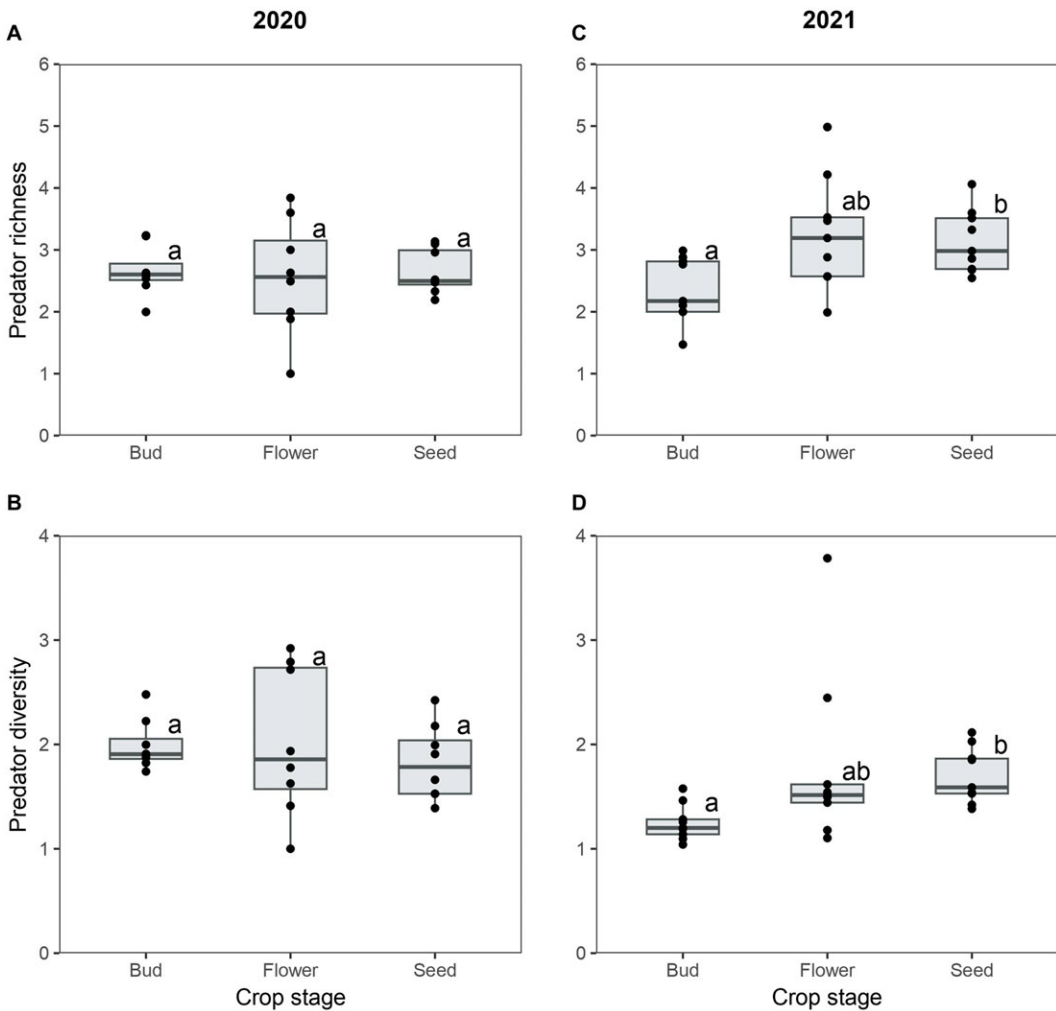


Figure 3. Rarefied richness and diversity (genus level) of generalist predators sampled at three alfalfa crop stages during 2020 and 2021: **A**, predator richness in 2020; **B**, predator diversity in 2020; **C**, predator richness in 2021; and **D**, predator diversity in 2021. Rarefactions were based on twice the lowest sample size per year (8 and 24 individuals in 2020 and 2021, respectively). Letters indicate statistical differences between values at each crop stage based on Conover tests, with *P*-values adjusted with the Benjamini-Hochberg method.

Abundance correlations between pests and natural enemies

We found a number of positive correlations between pests and natural enemy abundances (Table 2). In 2020, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and Coccinellidae were positively correlated with *Lygus* spp. Also in 2020, Araneae were positively correlated with *A. lineolatus*. In 2021, *Nabis* spp. was positively correlated with *Lygus* spp. We found no correlations between *H. postica* larvae and either *B. curculionis* or *O. incertus* in either year.

Discussion

Characterising the seasonality of insect pests and their natural enemies, and identifying the correlations occurring between them, can provide important foundational knowledge for the

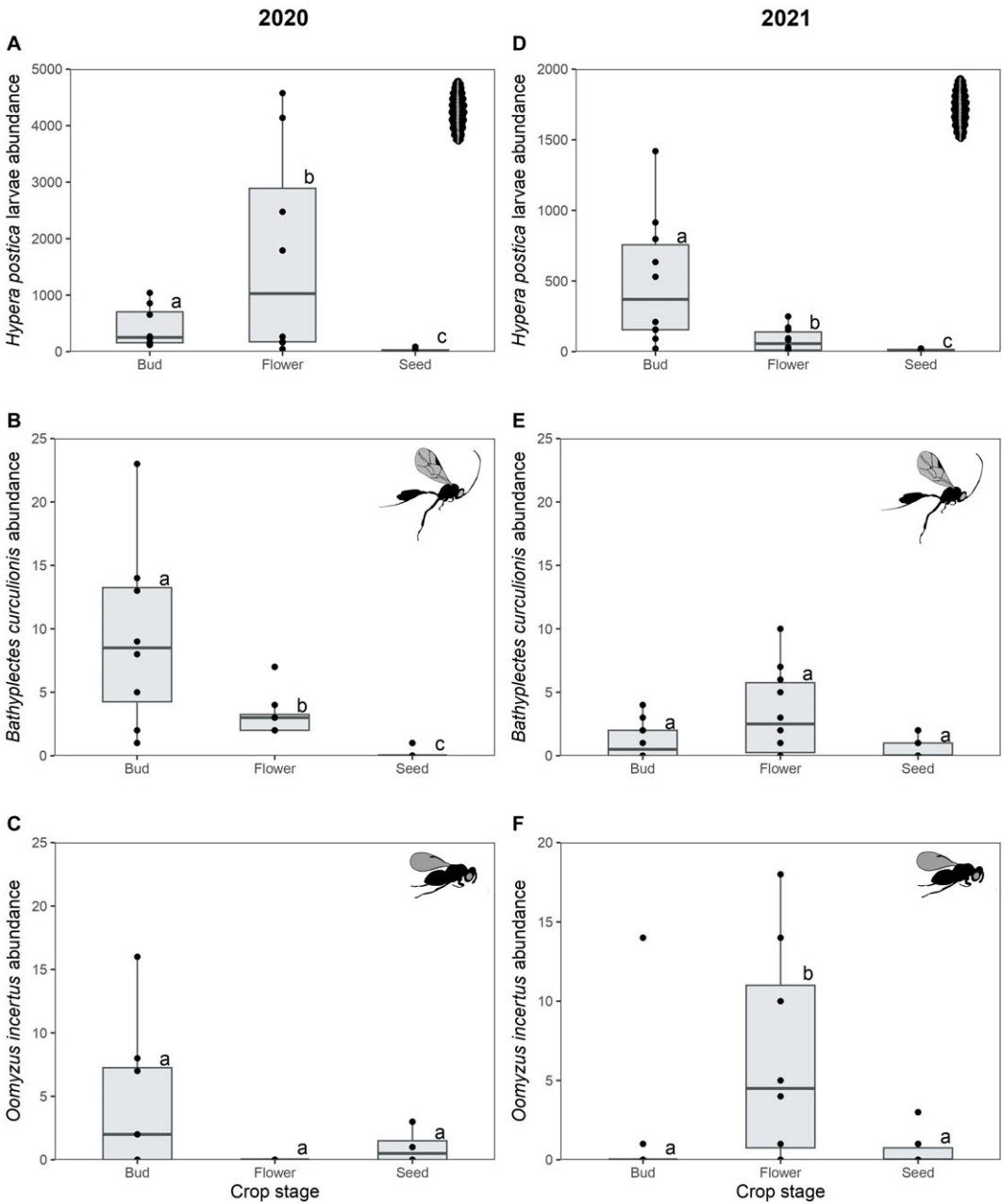


Figure 4. Abundance of *Hypera postica* larvae and its parasitoids, *Bathyplectes curculionis* and *Oomyzus incertus*, sampled at three alfalfa crop stages in 2020 and 2021. Values indicate individuals collected per 100 sweeps: **A**, *H. postica* larvae in 2020; **B**, *B. curculionis* in 2020; **C**, *O. incertus* in 2020; **D**, *H. postica* larvae in 2021; **E**, *B. curculionis* in 2021; and **F**, *O. incertus* in 2021. Letters indicate statistical differences between values at each crop stage based on Conover tests, with *P*-values adjusted with the Benjamini–Hochberg method.

Table 2. Repeated measures correlations between pests and natural enemies in alfalfa seed production fields in southern Alberta in 2020 and 2021. The *P*-values are adjusted with the Benjamini–Hochberg method (*, **, and *** indicate significant correlations at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively)

Natural enemies	Pests							
	<i>Adelphocoris lineolatus</i>		<i>Lygus</i> spp.		<i>Hypera postica</i> adults		<i>Hypera postica</i> larvae	
	2020	2021	2020	2021	2020	2021	2020	2021
<i>Nabis</i> spp.	0.35	0.55	0.35	0.73**	0.62	0.04	-0.5	-0.37
<i>Orius</i> spp.	-0.08	-0.01	0.27	0.42	0.2	0.13	-0.43	-0.31
<i>Chrysoperla</i> spp.	0.07	-0.11	0.79**	0.13	0.01	0.15	-0.27	-0.32
Coccinellidae	0.14	-0.02	0.66*	0.28	0.04	0.01	-0.13	-0.27
Araneae	0.80**	0.16	0.19	0.37	0.01	0.49	0.10	-0.36
<i>Bathyplectes curculionis</i>	-	-	-	-	-	-	-0.03	-0.07
<i>Oomyzus incertus</i>	-	-	-	-	-	-	-0.32	-0.08

development and implementation of conservation and integrated pest management strategies. In this study, we examined the presence and abundance of a selected guild of insect pests and their natural enemies across six arthropod orders from alfalfa seed fields in southern Alberta and investigated their correlations throughout the growing season.

Seasonal abundance of *Lygus* spp. and *A. lineolatus*

In the present study, we observed inconsistent seasonal abundance trends for *Lygus* spp. across years. *Lygus* spp. abundance did not differ between crop stages in 2020. However, in 2021, *Lygus* spp. abundance differed between crop stages and increased continually throughout the season. The peak in *Lygus* spp. abundance that we observed in 2021 is consistent with the second-generation adult peaks in mid-August that have been reported in Manitoba (Uddin 2005), Alberta (Butts and Lamb 1991), and Saskatchewan (Braun *et al.* 2001), but we did not observe this pattern in 2020. Inconsistent seasonal abundance trends may be attributed partially to differences in climate between years, among other environmental and biotic factors. In 2021, high summer temperatures (discussed below, in the Discussion section) and drought likely allowed *Lygus* populations to increase locally and may also have resulted in higher migration to irrigated seed alfalfa fields from drought-affected crops. Insecticide applications also may have varied between years, impacting *Lygus* dynamics. However, our data do not allow us to discuss this further. In contrast to *Lygus* spp. abundances, we observed somewhat consistent seasonal abundance trends for *A. lineolatus* in 2020 and 2021, with peaks occurring during the bud and seed stages in both years. This result concurs with observed abundance peaks reported by Uddin (2005) in Manitoba in mid-June and later again in mid-August. Even though *A. lineolatus* is mostly considered univoltine north of 51° N, two generations can occasionally occur, depending on temperature (Craig 1963). In agreement with Uddin's (2005) observations of a partial second generation in Manitoba, the relatively high numbers of *A. lineolatus* that we observed during the seed stage suggest a second generation and deserve further study. Sim and Meers (2017) noted that *Lygus* spp. and *A. lineolatus* tend to stay under the economic thresholds in alfalfa forage crops in southern Alberta. In accordance with this, over the two years of the present study, *Lygus* spp. and *A. lineolatus* abundances mostly remained under the economic threshold (2–3 late-instar larvae or adults per sweep for seed alfalfa; Government of Alberta 2024). Nonetheless, in 2021, adult

Lygus spp. densities reached economic thresholds (*i.e.*, > 200 individuals per 100 sweeps) during the seed stage in three of 10 fields (data not shown).

Predator seasonal abundance, richness, and diversity

During the present study, we documented the presence and seasonal abundance of 10 predatory arthropod taxa in alfalfa seed production fields in southern Alberta. The occurrence of these taxa in alfalfa fields concurs with the results of previous studies in alfalfa fields in the Canadian prairies (Table 1; Hobbs *et al.* 1959; Harper 1988; Uddin 2005; Sim and Meers 2017; Soroka *et al.* 2020). We found that the predator complex was dominated by *Orius* spp., *Nabis* spp., Coccinellidae, and Araneae, consistent with other studies conducted in the Canadian prairies (Schaber and Entz 1988) and the United States of America (Elliott and Kieckhefer 1990; Elliott *et al.* 2002; Pons *et al.* 2005, 2009; Rand 2017). Despite this, our data suggest considerable variation in seasonal abundance trends for predatory taxa between years. Based on data collected over 13 years, Elliott and Kieckhefer (1990) noted that natural enemy abundances in alfalfa fields in South Dakota, United States of America, fluctuate considerably from year to year and are often unrelated to co-occurring taxa and abundances in previous years. The inconsistent abundance trends we observed for predators across the two years of the present study can be attributed to multiple environmental and biotic factors, as well as to management practices such as insecticide applications. Climatic differences, landscape characteristics, resource availability, biotic interactions, and habitat disturbances can strongly influence natural enemy densities in agricultural fields (Chaplin-Kramer *et al.* 2011; Lantschner *et al.* 2019). The influence of these factors and particularly the timing and choice of insecticide application should be considered in the development of integrated pest management strategies and natural enemy population predictions.

In the present study, *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) strongly dominated the Coccinellidae assemblage. Following the introduction and establishment of *C. septempunctata* in North America, concern has been raised as to what appears to be a displacement of native Coccinellidae species by *C. septempunctata*, as evinced by decreasing lady beetle diversity (Alyokhin and Sewell 2004; Harmon *et al.* 2007). In an earlier survey, Harper (1988) recorded 22 different species of lady beetles in alfalfa fields in Alberta, all native to North America, whereas Sim and Meers (2017) recorded 12 species, with *C. septempunctata* dominating this group. In the present study, we documented six Coccinellidae species over two sampling years, of which five were native. Thus, results from the present study seem to be following a trend of decreasing lady beetle richness in alfalfa. However, differences in sampling effort between the present and earlier studies should be considered, and further research in this area is warranted.

Overall, in the present study, the richness and diversity of sampled predators varied over time, with inconsistent patterns occurring between years. Predator richness stayed constant across crop stages in 2020, but interestingly, in 2021, it increased throughout the season and peaked during the seed stage. Similarly, predator diversity remained constant in 2020 but increased throughout the season in 2021, again peaking during the seed stage. Although natural enemy diversity is generally considered beneficial in terms of biological control, this relationship is a subject of debate in the literature (Jonsson *et al.* 2017); further research should explore the effects of natural enemy diversity on pest populations in alfalfa fields. In addition, because we did not identify Araneae and juvenile lady beetles to genera, we likely underrepresented the true species richness and diversity of predators in the study fields.

Seasonal abundance of *H. postica* larvae, *B. curculionis*, and *O. incertus*

We observed variable trends in the abundance of *H. postica* larvae and its parasitoids, *B. curculionis* and *O. incertus*, between collection years. Although we observed a peak in *H. postica*

larval abundance at the flower crop stage in 2020, this peak occurred during the bud stage in 2021. Observed peaks in *H. postica* larval abundance, however inconsistent, seem to fall between those reported by Soroka *et al.* (2020) in Saskatchewan as occurring between mid-June and early July. The inconsistent trends in *H. postica* larvae observed in the present study may be due in part to differences in temperature between years. This factor is critical for *H. postica* overwintering and spread (Soroka *et al.* 2020): survival, longevity, and reproduction of *H. postica* greatly decrease at temperatures rise above 32 °C (Levi-Mourao *et al.* 2022). During the present study, 2021 was much warmer than 2020: June and July 2020 had averages of 19.0 °C and 22.6 °C and high extremes of 25.8 °C and 29.1 °C, respectively, whereas June and July 2021 had averages of 23.7 °C and 25.4 °C and extremes of 35.0 °C and 35.5 °C, respectively (Government of Canada 2022). Extreme temperatures therefore may have depressed *H. postica* populations in 2021. In addition to between-year temperature differences, crop phenology differed between 2020 and 2021. Although collection dates at the bud stage occurred six calendar days apart in 2020 relative to 2021 (from 10 to 12 June *versus* 16 June, respectively), and only one day apart at the seed stage (from 10 to 11 August 2020 *versus* 11 August 2021), collection dates at the flower stage occurred almost two weeks apart in 2020 compared to 2021 (from 4 to 6 July *versus* 21 July, respectively). We suspect that the higher temperatures seen in 2021 may have accelerated crop development in July, further affecting *H. postica* larval abundance. We observed the presence of *H. postica* larvae throughout the entire season during both years of this study. Despite this species being univoltine in Canada (Bereza 1970; Soroka *et al.* 2020), *H. postica* larvae seem to be present throughout the entire season in the Canadian prairies (Soroka *et al.* 2020). Nonetheless, larval abundance decreased dramatically by the seed stage, consistent with Soroka *et al.*'s (2020) results.

In the present study, we collected *B. curculionis* throughout the entire growing season in both years. Although *B. curculionis* is documented to have a partial second generation in some areas of the United States of America (Chamberlin 1926; Radcliffe and Flanders 1998), the species is univoltine in Canada (Soroka *et al.* 2020), even though, like its host, it remained present throughout the season. *Bathyplectes curculionis* peak flight occurs in mid-June, 1–2 weeks before *H. postica* larval populations peak (Soroka *et al.* 2020). In the present study, we observed a peak in *B. curculionis* abundance in mid-June (flower stage) in 2021 but not in 2020.

Oomyzus incertus is documented to have 3–4 generations per year in the United States of America, with peak abundances generally occurring in mid-summer, depending on the region (Radcliffe and Flanders 1998). In the Canadian prairies, *O. incertus* is multivoltine and persists until late August (Soroka *et al.* 2020); however, the exact number and timing of peak flights are unknown in Alberta because this species is considered recently established in the Canadian prairies (Soroka *et al.* 2020). In 2020, no individuals of *O. incertus* were collected during the flower stage, in contrast to 2021, when *O. incertus* abundance peaked during the flower stage. The varying numbers of *O. incertus* collected during the two years of this study and the lack of previous information on population peaks in southern Alberta make drawing conclusions on the species' seasonal abundance difficult. Given the differences in abundance and population trends across years and locations, more research investigating the life cycles and seasonal abundance of *B. curculionis* and *O. incertus* over a longer time span is needed to assess the species' potentials as biocontrol agents for *H. postica* in southern Alberta.

Abundance correlations between pests and natural enemies

During the two years of our study, we found positive correlations between alfalfa pests and some generalist predators. Although our findings do not reveal trophic interactions between species and any correlations may result from shared responses to environmental factors, such correlations have biological implications and may indicate numerical responses to pest densities. First, we observed a positive correlation between *C. carnea* and *Lygus* spp. in 2020. This result is consistent with Schaber and Entz (1988), who found a positive correlation between lacewings and

Lygus spp. abundances, also in southern Alberta. In addition, molecular gut content analyses show evidence of light predation on *Lygus* spp. by *C. carnea* under controlled conditions and in alfalfa as a trap crop in California, United States of America (Mansfield and Hagler 2016; Hagler *et al.* 2018). Second, we observed a positive correlation between Coccinellidae and *Lygus* spp. abundances in 2020, concurring again with Schaber and Entz (1988) but contrasting with Uddin (2005), who found a negative relationship between Coccinellidae and *Lygus* spp. abundances in alfalfa fields in Manitoba. Mansfield and Hagler (2016) report predation and scavenging on *L. hesperus* by *H. convergens*. Nonetheless, *C. septempunctata*, the dominant lady beetle species collected in our study, did not consume *L. lineolaris* in controlled feeding experiments by Arnoldi *et al.* (1991) and displayed low consumption rates on *L. hesperus* in a cage experiment by Hagler *et al.* (2020). This indicates that further research is required to assess whether Coccinellidae assemblages in southern Alberta alfalfa feed on *Lygus* spp. Third, we identified a positive correlation between Araneae and *A. lineolatus* in 2020. This correlation agrees with correlations noted by Schaber and Entz (1988), and Howell and Pienkowski (1971) observed evidence of predation on *Adelphocoris* sp. Reuter by spiders in the families Thomisidae, Salticidae, Lycosidae, and Tetragnathidae under controlled conditions. However, we are not aware of field studies that investigate trophic interactions between these taxa. Finally, we observed a positive correlation between *Nabis* spp. and *Lygus* spp. in 2021. Previous research by Schaber and Entz (1988) supports this finding, and feeding experiments by Arnoldi *et al.* (1991) and Clancy and Pierce (1966) also show relatively high incidences of *Nabis* spp. feeding on *L. lineolaris* and *Lygus hesperus* Knight (Hemiptera: Miridae). Moreover, molecular gut content analysis shows that *N. alternatus* Parshley (Hemiptera: Nabidae) feeds on *L. hesperus* (Hagler *et al.* 2018) in alfalfa trap crops in California, and *N. americanoferus* Carayon (Hemiptera: Nabidae) successfully reduced *L. lineolaris* populations in a release experiment in strawberry (Rosaceae) fields in Québec (Dumont *et al.* 2023). Given the correlations we noted between pests and natural enemies in the present study, further research is needed to determine the effects of these generalist predators on pest populations.

We did not observe correlations between Araneae and *Lygus* spp. abundances in either year, despite evidence from Arnoldi *et al.* (1991), Hagler *et al.* (2018), and Hagler *et al.* (2020) of predation on *Lygus* spp. by spiders in the families Agelenidae, Philodromidae, and Thomisidae under controlled and field conditions. Similarly, we did not find correlations between *Orius* spp. and *Lygus* spp. in either year, despite evidence by Hagler *et al.* (2018) and Dumont *et al.* (2023) of predation on *Lygus* spp. by *Orius tristicolor* White (Hemiptera: Anthocoridae) and *O. insidiosus* in the field.

Even though we did not find significant correlations between *H. postica* larval abundances and any of the predators we sampled, we noted that relationships between these taxa seem to follow a negative direction. This observation agrees with Schaber and Entz (1988), who found only negative correlations between predators and *H. postica* in southern Alberta. Nonetheless, Rand (2017) documented aggregation responses of Nabidae and Coccinellidae to *H. postica* in Montana and North Dakota, United States of America. We should note, however, that given the cyclic nature of prey–predator dynamics (Crawley 1975), the direction of correlations between two taxa may potentially differ according to the time of sampling. Interestingly, Evans and England (1996), Elliott *et al.* (2002), and Batuecas *et al.* (2022) provide evidence of predation on *Hypera* spp. by *Orius* spp. and Coccinellidae in Spain and Utah, United States of America, respectively, and Ouayogode and Davis (1981) suggest evidence of predation on *H. postica* by Coccinellidae, *Chrysopa oculata* (Say) (Neuroptera: Chrysopidae), *Nabis* spp., and *Xysticus* sp. Koch (Araneae: Thomisidae) in laboratory feeding trials. Therefore, the potential trophic interactions between generalist predators and *H. postica* in alfalfa fields in Alberta should be investigated.

We found no correlations between *H. postica* larvae and *B. curculionis* or *O. incertus* in either 2020 or 2021. In North Dakota and Montana, United States of America, Rand (2013) correlated *B. curculionis* parasitism rates to alfalfa weevil larval densities, even though the direction of these

correlations changed from a positive to negative in a two-year span, which might be attributed to environmental factors.

In the present study, we did not quantify aphids in the field, although aphid abundances likely would influence predator populations: Pons *et al.* (2005, 2009) noted evidence of predation and numerical responses to aphids by Coccinellidae, Chrysopidae, and Nabidae. In addition, a positive correlation may exist between aphid abundance and parasitism of *H. postica* by *B. curculionis* that could be due to honeydew consumption by the parasitoid (Evans and England 1996; Rand and Lundgren 2019), which deserves attention. Although we requested insecticide application data for the fields we sampled, we obtained only a partial dataset and had insufficient field replication to investigate the effect of insecticide applications on pest and natural enemy populations. Chemical insecticides have been shown to affect both pest and natural enemy populations in alfalfa (Harper 1978), and recommendations on insecticide use in alfalfa have been made in relation to crop phenology, pest populations, and some beneficial insects occurring in fields (Soroka *et al.* 2011). Lastly, we focused on only a few of the pests that are present in alfalfa fields. This limitation affected our observations, and future investigations into other pests in alfalfa (e.g., alfalfa seed chalcid, aphids, and cicadellids) should be considered.

Here, we have documented seasonal abundance of three pests and the seasonal abundance, richness, and diversity of generalist predators in alfalfa fields in southern Alberta during two collection years. Although some of our abundance observations concur with other studies, we observed important inconsistencies in trends between years, which need to be addressed further in future studies to improve understanding of the effects of environmental conditions on abundance patterns. Moreover, the life cycles of *H. postica* parasitoids in alfalfa fields in relation to host abundance patterns in southern Alberta require further investigation. In addition, the potential occurrence of a second-generation of *A. lineolatus* in southern Alberta requires further research. This information is essential to guide growers towards improved management strategies to conserve natural enemies and reduce dependency on insecticides. The trophic interactions between generalist predators and alfalfa pests under a biological control framework in southern Alberta also present ample opportunity for further research.

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