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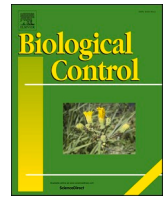Kristopher L. Giles

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Role of parasitoids and landscape structure in aphid population dynamics in winter canola

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HIGHLIGHTS

- Two parasitoids with broad host ranges, *Diaeretiella rapae* (M'Intosh) and *Aphelinus nigritus* (Howard), parasitized aphids in central Oklahoma canola fields.
- Aphid parasitism by *D. rapae* was positively associated with characteristics of the landscape surrounding canola fields, particularly the amount of landcover by wetlands.
- Parasitism by *A. nigritus* was positively but weakly associated with landcover of summer crops, but not by other measured landscape variables.
- There were fewer aphids and greater parasitism of aphids in field cages that allowed access by parasitoids than in cages that excluded natural enemies.
- Guidelines for habitat management to increase biological control efficacy, guidelines for assessing potential for biological control based on knowledge of landscape structure, or both are potential outcomes of the research.

ARTICLE INFO

ABSTRACT

Three aphid species infest winter canola, *Brassica napus* L. fields in central Oklahoma and are serious pests: the cabbage aphid, *Brevicoryne brassicae* L., green peach aphid, *Myzus persicae* (Sulzer), and turnip aphid, *Lipaphis pseudobrassicae* (Davis). Mortality caused by parasitoids may be an important component of biological control of aphids in Oklahoma canola fields. Therefore, it is important to determine the effect of parasitoids on aphids in canola and the factors that affect it. We undertook a study during three consecutive growing seasons to: 1) estimate aphid suppression by parasitoids in each of 23 canola fields using cages that excluded natural enemies and cages that permitted access by parasitoids; and 2) evaluate how aphid parasitism in canola is related to the composition and configuration of the landscape surrounding each canola field. Parasitism was estimated using turnip aphid infested sentinel canola plants stationed in each field in autumn and in spring of each growing season. Two parasitoids with broad host ranges, *Diaeretiella rapae* (M'Intosh) and *Aphelinus nigritus* (Howard), parasitized turnip aphids in canola. There were fewer aphids and more parasitoids in cages that permitted access to parasitoids than in cages that excluded natural enemies. Partial redundancy analysis demonstrated that parasitism rate by *D. rapae* was positively related to landcover of wetlands and negatively related to contagion of patches. Parasitism by *A. nigritus* was positively but weakly associated with landcover of summer crops, but not with any other measured landscape variable. Wetlands in central Oklahoma agricultural landscapes apparently are habitat for aphid parasitoids from which they disperse to canola fields and parasitize aphids. Partial redundancy analysis showed that relative aphid density in fields was negatively related to parasitism by *D. rapae*. Results indicate that parasitoids suppress aphid infestations in canola fields and demonstrate potential for habitat management to improve biological control of aphids in canola.

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1. Introduction

During the last 20 years, winter canola (*Brassica napus* L.) has become an important winter crop in Oklahoma. Winter canola typically is planted during October, grows through autumn and spring, and is harvested in June. Canola was introduced as a rotational crop with winter wheat (*Triticum aestivum* L.) to improve weed management capability, reduce plant disease pressure in continuous wheat, and provide an alternate cash crop. There often are increased profits from wheat-canola rotation compared to wheat only production (Bushong et al. 2012). In the U.S., canola produces high oil yields and is profitable without subsidies giving it potential as a biodiesel crop (Smith et al. 2007, Dansby 2008).

Several aphid species infest canola fields in this region including the cabbage aphid, *Brevicoryne brassicae* (L.); green peach aphid, *Myzus persicae* (Sulzer); and turnip aphid, *Lipaphis pseudobrassicae* (Davis) (Elliott et al. 2014) and are serious pests often requiring insecticide application to protect yields (Royer and Giles 2017). In winter wheat, a well studied crop inOklahoma, mortality caused by parasitoids is important to aphid biological control (Giles et al. 2003, Elliott et al. 2018) and understanding the role of parasitoids in aphid population dynamics in canola fields is essential to gauging their potential for aphid biological control in winter canola.

The loss of and fragmentation of natural habitats is extensive in Oklahoma, and agroecosystems are dominated by winter wheat, managed grassland (pasture, hay, and rangeland), and food, fiber, and fuel crops, such as canola, corn (*Zea mays* L), cotton (*Gossypium hirsutum* L.), soybean (*Glycine* max (L.) Merr.), and sorghum (*Sorghum bicolor*, (L.) Moench). Winter canola fields in Oklahoma are typically interspersed in a landscape consisting of fields of winter wheat, pasture, summer crops, fallow fields, and semi-natural habitats such as wetlands and woodlands.

French et al. (2001) observed that the parasitoid *Diaeretiella rapae* (M'Intosh) occurred in canola in Oklahoma and was the dominant parasitoid in the crop, with the only other parasitoid recovered from aphids in canola being *Lysiphlebus testaceipes* (Cresson), which was reared infrequently from *L. pseudobrassicae* mummies from canola but not from *B. brassicae or Myzus persicae* mummies. Boyd and Lentz (1994) in a field study in western Tennessee (ca. 500 km east of our study area) found that *D. rapae* was the only aphid parasitoid in canola. *Diaeretiella rapae* was the only parasitoid consistently reared from aphids collected from canola fields in central Oklahoma other than a single specimen of an unknown species of *Aphelinus* (possibly *Aphelinus asychis* Walker) that was reared from *B. brassicae* (Elliott et al. 2014). Sorghum can remain green and can host aphids until the first hard freeze in autumn and *Aphelinus nigritus* Howard adults apparently spill over from sorghum to wheat and canola (Giles et al. 2021). The limited information available on the aphid parasitoid fauna in canola in central Oklahoma suggests that there are only the two above mentioned species with *D. rapae* being the dominant of the two.

The spatial and temporal configuration of landscape elements that contribute to agroecosystem diversity also likely influence biological control effectiveness by determining the availability, number, and distribution of hosts for aphid parasitoids in the landscape. Because *D. rapae* parasitizes a wide range of aphid species in several habitats (Pike et al. 1999) its population density and foraging activity in canola fields may depend on the presence, abundance, and spatial distribution of habitats in the surrounding agricultural landscape that contain hosts for *D. rapae*. In addition to hosts resources such as food, which markedly affect longevity of adult *D. rapae* (Jamont et al. 2013), vary spatially and temporally in central Oklahoma agricultural landscapes (Elzay and Baum 2021). The overwintering biology of *D. rapae* in central Oklahoma has not been studied, but comparison to overwintering by *D. rapae* in Europe (Geiger et al. 2005) and to *Lysiphlebus testaceipes* (Cresson), which has been extensively studied in Oklahoma (Giles et al. 2003), suggests that *D. rapae* overwinters within canola fields and other habitats maintaining hosts, and may or may not enter diapause during

winter. The proximity of canola fields to habitats providing hosts and other resources may influence parasitism levels in canola.

Diaeretiella rapae has a broad host and habitat range (Mackauer and Stary 1967, Stary 2006). However, recent research has indicated that *D. rapae* may be more accurately described as being comprised of multiple biotypes each with limited host range (Antolin et al. 2006, Navasse et al. 2018, Derocles et al. 2020). If this is true for *D. rapae* parasitizing aphids in canola the effect of alternate habitats on parasitism of aphids in canola would be expected to be limited. This might be exemplified by semi-natural habitats where Navasse et al. (2018) observed lack of host switching by *D. rapae* from aphids on the non-cultivated plant Lamb's Quarter, *Chenopodium album* L., to aphids on cultivated Cruciferae.

There are no cruciferous crops other than canola commonly grown in central Oklahoma, but there are over 60 species of wild crucifers in Oklahoma (Folley 2011) some of which are common (e.g. wild mustard, *Brassica haber* (DC.)). Aphid pests of canola occur on non-cultivated mustard species that grow in farm fields, roadsides, pastures, and semi-natural lands (Tamaki 1975, Horn 1981). Wild mustards grow in a variety of habitats and soil types in Oklahoma and elsewhere in the Plains States and are present in cultivated fields, grasslands, riparian areas, wetlands, roadsides, and disturbed sites (Folley 2011). Some wild Cruciferae species may be higher quality hosts for aphid species such as *M. persicae* than canola (Le Guigo et al. 2012), and therefore presumably higher quality resources for *D. rapae*. The role wild crucifers may play in hosting aphids and *D. rapae* is unknown.

The biology and ecology of *Aphelinus nigritus* is not well known. The species is known to parasitize aphids in sorghum and wheat in the U.S. Southern Plains (Langston 1970, Kring and Gilstrap 1983, Gilstrap et al. 1984). Until recently *A. nigritus* was uncommon in wheat and sorghum and was not found in canola (Kring and Gilstrap 1983, Gilstrap et al. 1984, Elliott et al. 2014). However, with invasion of sugarcane aphid, *Melanaphis sorghi* (Theobald) onto the Southern Plains in 2013 (Bowling et al. 2016) abundance of *A. nigritus* on sorghum has increased (Maxon et al. 2019), which appears to have resulted in spillover of the species into winter crops grown in central Oklahoma (Giles et al. 2021). Adult *Aphelinus nigritus* were collected from canola fields in Oklahoma in a recent quantitative study of aphid natural enemies in the crop (Elliott et al. 2023), suggesting that *A. nigritus* might be parasitizing aphids in canola.

We had two objectives for this study. First, to determine if parasitoids reduced aphid populations in canola fields. For this objective, we estimated aphid numbers in aphid infested cages that excluded natural enemies compared to aphid infested cages that allowed access by aphid parasitoids. Our second objective was to determine if parasitism of aphids in canola fields in central Oklahoma was dependent on the composition and configuration of the landscape matrix within which the fields were embedded and to identify attributes of landscape structure that influenced parasitism rates. To accomplish this objective, we measured compositional and configurational attributes of the landscape matrix surrounding study canola fields and compared that to aphid parasitism on sentinel plants stationed in the fields.

2. Materials and methods

2.1. Study fields

Study fields were selected for study in autumn when winter canola is planted which will be harvested in late spring of the following year. In this manuscript we refer to the growing season of the crop by the year it was planted i.e., the 2016 growing season (seven fields) and the 2017 and 2018 growing seasons (eight fields in each) (Fig. 1). Fields each growing season were located at least 5 km apart. Management practices used in each field were those selected by the grower. All fields were planted with insecticide treated seed, which is a nearly universal practice in Oklahoma canola production. Neonicotinoid insecticide treated seed is usually planted, but we did not confirm with growers which

insecticide chemical group was used to treat seed. The study protocol was repeated twice each growing season in each field, once in early November 2–3 weeks after canola plants emerged from the soil and were in the seedling to rosette stage and a second time in mid-March when plants were in the budding through bolting stages.

2.2. Experimental layout for aphid sampling and sentinel plants

Study fields were sampled twice each growing season for three growing seasons. Each field was sampled in early November and again in mid-March. Sampling, in each field was accomplished within an area of approximately 100x70 m (Fig. 2). A backpack Model 24 D-Vac® (Rincon-Vitova Insectaries, Inc., Ventura, CA) suction sampler fitted with a 33 cm diameter collecting head, organdy collecting bag, and fiberglass collar was used to sample aphids. A subsample consisting of 20 placements of the D-Vac collection unit was taken along each of three parallel transects each 100 m long for a total of 60 placements per field. The total area sampled in 60 placements was 5.15 m^2. Transects were spaced 25 m apart, perpendicular to the nearest field edge, and approximately 25 m into the field from the edge. Every 5 m along each transect the D-vac collecting head was placed straight down over growing canola plants to just above the soil surface and held there for 5 s. This was repeated along the transect until 20 placements had been made. After 20 placements the sampling bag was removed from the D-vac and the contents were put into a labeled plastic bag. Plastic bags were transported to the laboratory and frozen until they could be processed. The aphids and adult parasitoids in each bag were counted. Parasitoids were identified to species, but aphids were only counted. Counting was considered sufficient for aphids because turnip aphid is the only aphid present on canola in appreciable numbers in central Oklahoma during vegetative growth stages of canola (Royer and Giles 2017).

In October of each year and again in February canola seeds were planted in 80 15 cm diameter plastic pots (ca. 7 seeds per pot). Each pot was caged with a 12 cm diameter by 35 cm height plastic cage and maintained in a greenhouse for ca. 3 weeks at which time approximately 30 turnip aphids were placed on plants in each caged pot. We chose turnip aphid to infest sentinel plants and exclusion cages (see below)

because this species is consistently present in canola during vegetative growth stages (autumn through early spring) in central Oklahoma. Green peach aphid and cabbage aphid were not good choices for sentinel plants because green peach aphid is sporadic and usually present during reproductive growth stages later in spring, while cabbage aphid is not present in appreciable numbers until reproductive stages of the crop (Royer and Giles 2017). Therefore, infesting with turnip aphid most closely mirrors species occurrence in canola fields during autumn and early spring in central Oklahoma. In early November nine potted aphid infested canola plants were placed in each study field. Potted plants were placed in the field approximately 10 days after infesting with aphids, at which time there were approximately 300 parasitoid-free aphids per plant. Three sentinel plants were stationed approximately equidistant adjacent to each of the three aphid sampling transects for a total of nine sentinel plants stationed in each field (Fig. 2). Plants were left in the field for three days and then caged and returned to a greenhouse where they were maintained for seven days to allow parasitoids to develop to the pupal stage. After seven days the plants from each pot were cut, placed in an emergence canister, and held for 14 or more days to allow adult parasitoids to emerge. Adult parasitoids in each emergence canister were counted and identified to species and intact braconid and aphelinid mummies counted. The number of parasitoids of each species per sentinel plant provided an estimate of the amount of parasitism.

Eight plants were arbitrarily selected from among the 80 caged plants and were maintained in the greenhouse rather than stationed in fields. These plants were processed identically to field stationed sentinel plants and served as checks that plants had not been contaminated with parasitoids in the greenhouse prior to field release which, if it occurred, would invalidate estimates of parasitism from field stationed sentinel plants. No parasitoids were found on check plants during the study.

2.3. Exclusion cages

An exclusion cage study was conducted in each of the canola fields in (mid-March) of each growing season. Extended heavy rains in spring of 2019 delayed sampling from within cages and most of the caged plants
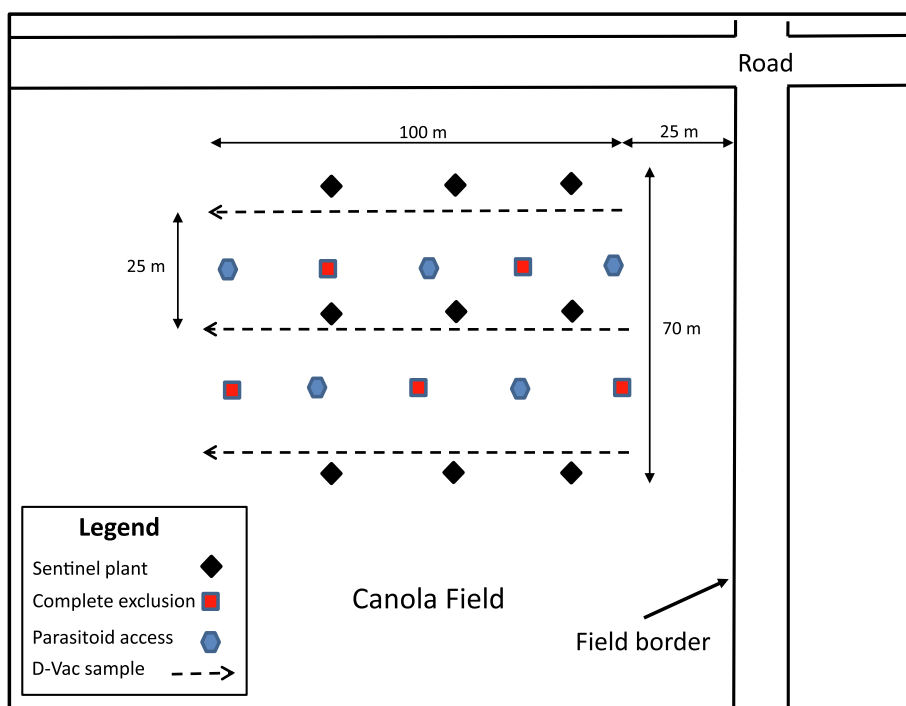

Fig. 1. Map of Oklahoma state showing locations of canola fields in which the study was accomplished during the 2016, 2017, and 2018 canola growing seasons.
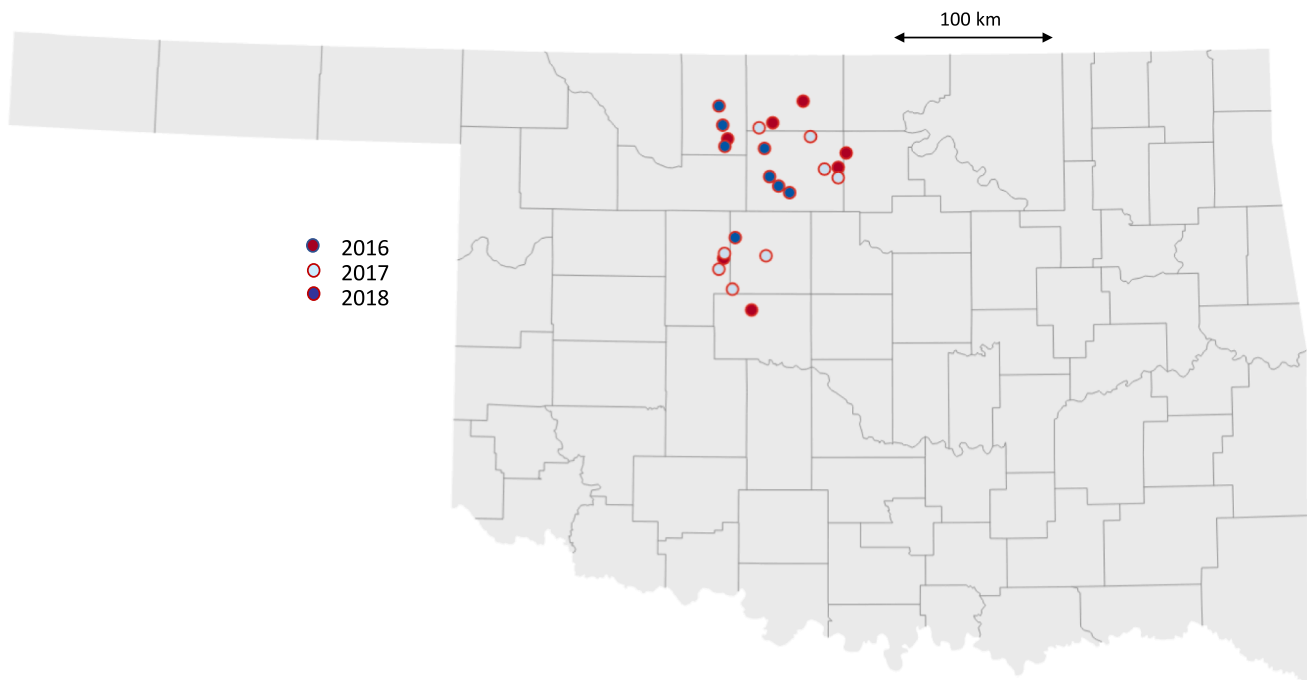
Fig. 2. Schematic diagram of exclusion cage positions, sentinel plant locations, and D-Vac suction sampling transects for a hypothetical canola field. Not drawn to scale.

died prior to sampling. Therefore, only data from 2016 and 2017 were analyzed. In 2016 cages in one field were severely damaged by a windstorm and their contents compromised. Thus, data from only six fields were used in 2016. The studies ran for approximately four weeks. Enclosures used in the experiments were mesh material tubes of 0.35 m diameter and 1.25 m length. Tubes were fitted over frames constructed from two u-shaped pieces of 0.95 cm diameter rebar. Tubes were tied into a knot at the top and buried by soil at the bottom to ensure closure. Two cage types were used: 1) A fine mesh enclosure made of polyester organza with approximately 2500 cells/cm^2, which prevented insects from entering (complete exclusion); and 2) A coarse mesh enclosure made of Lumite® (Lumite Inc., Alto, GA) screening with 64 cells/cm^2, which was coarse enough to allow entry by aphid parasitoids but not by most predatory arthropods (partial exclusion). A single canola plant growing in the field that appeared to be growing normally was enclosed by each cage. Five cages of each type were positioned 20 m apart along two 80 m transects in each field starting ca. 25 m from the field edge (Fig. 2).

Once exclusion cage frames were established in the field, the plant and soil surface in each cage were treated with insecticide, Pyrethrin (Bonide Products Inc., Oriskany, NY) 12 gm/l, which was applied to the leaves and stems using a handheld sprayer to kill arthropods that might be present on the plants. Then the mesh cages were installed as described above. Approximately seven days after pyrethrin application each cage was infested with ca. 100 turnip aphids from a greenhouse maintained colony. After four weeks 12 leaves were carefully cut from each caged canola plant, placed in a labeled plastic bag, and transported to the laboratory. Due to time constraints samples were frozen at the laboratory and insects were counted at a later date. We counted the numbers of aphids and braconid and aphelinid mummies on the 12 leaves from each cage. Parasitoid mummies were sometimes found in samples from complete exclusion cages indicating unexpected entry of parasitoids into them. Mummies were often found in more than one complete exclusion cage within the same field and always in numbers several times smaller than in partial exclusion cages from the same field. Cages sometimes developed small holes caused by insects such as grasshoppers or other small animals or by mechanical abrasion, which

were probably the source of contamination. Because of the much smaller number of mummies in complete exclusion cages compared to partial exclusion cages we chose to analyze data from all cages, with the understanding that differences in aphid and parasitoid numbers among cage types would be biased toward showing a smaller effect of parasitoids on aphids among cage types than had actually occurred. The difference in the total number of aphids per cage for the five full exclusion and five partial exclusion cages from each field provided an estimate of aphid suppression by parasitoids.

2.4. Landscape measurements

Landscape context for each field was quantified for each of three circular areas centered on each study canola field with radii of 0.5, 1.5, and 2.5 km extracted from USDA NASS Cropland Data Layer (USDA, NASS 2015) for the appropriate year. The cropland data layer is a land use classification that focuses primarily on differentiating crop types with accuracy rates 85% or greater (https://www.nass.usda. gov/Research_and_Science/Cropland/sarsfaqs2.php date accessed 01/ 03/2022). The radii selected encompassed the likely average dispersal capabilities of aphid parasitoids (Thies et al. 2005, Elliott et al. 2018). The cropland data layer was re-classified to retain eight land cover categories: canola, fallow land, grassland (managed and unmanaged), summer crops (primarily corn, soybean, sorghum, and cotton), wetlands, wheat, woodlands, and other land uses (mostly developed land and roads). Aggregating land cover into fewer categories than in the NASS data was desirable for calculating meaningful landscape metrics because some categories would have been represented by very small areas and hence metrics would be subject to high variability. We quantified landscape structure for the circular area surrounding each field in each year using the following landscape metrics: the proportion of the total area in each land use type, patch density, fractal dimension, patch diversity (Shannon-Wiener index) (SHDI), and contagion (see McGarigal and Marks 1995, McGarigal 2014). The latter four metrics have straight-forward interpretations and the last three of these were found by Ritters et al. (1995) in a study of several landscapes to be good quantitative descriptors of landscape structure that were relatively

independent of one another. The metrics often have ecologically meaningful interpretations (O'Neill et al. 1988). Patch density measures the number of patches per unit area (km² by default) in a landscape and therefore implicitly measures average patch size. Average patch size and patch distribution can affect the connectivity of the landscape for a species. Connectivity is a species specific trait that is influenced by both the mobility and behavior of a species (Turner et al. 2001). The perimeter to area fractal dimension is dimensionless and increases with increasing patch boundary curvilinearity. Irregular shaped boundaries typify landscapes with many natural (non-human managed) patches for example riparian corridors (Turner et al. 2001). Contagion measures the amount of clumping of patch types in a landscape as a percentage of the maximum. Maximum contagion for a given landscape occurs when each landcover type is present as a single contiguous patch. High contagion indicates highly aggregated and poorly interspersed patches (McGarigal and Marks 1995). SHDI measures landscape composition, not configuration. Large values of SHDI indicate a large number of landcover types present in a landscape (in our case a maximum of eight), greater evenness in amount of each landcover type, or both (McGarigal and Marks 1995).

GIS operations were accomplished using ARCGIS® version 10.6 including exporting data for calculating landscape metrics using Fragstats. Landscape metrics were calculated using Fragstats Version 4®. The radius selected was based on inspection of pairwise correlations of parasitism rate to landscape metrics. The radius with the greatest number of correlations of parasitism to landscape metrics with P < 0.15 across all metrics and seasons was selected for use (see Table S1). Based on this preliminary inspection of correlations landscape data for a 1.5 km radius were selected for analyses.

2.5. Data analysis

PROC MEANS (SAS Institute 2015) was used to calculate descriptive statistics such as mean relative aphid density (number of aphids collected per 5.15 m² of D-vac sampling) and parasitism for various categories such as field, season, and growing season. Pearson correlations among variables of interest were calculated using PROC CORR (SAS Institute 2015).

PROC GLM (SAS Institute 2015) was used to test for effects of growing season, field, and exclusion cage type on the number of aphids per 12 leaf sample from each cage. The dependent variable, number of aphids per 12 leaves, was transformed to square root prior to analysis. A three factor hierarchical ANOVA model was fitted using PROC GLM in which the variable cage type (complete and partial exclusion) was nested within field and growing season and field was nested within growing season, i.e.

$$aphids^{1/2} = cagetype(field*gs) + field(gs) + gs + \varepsilon,$$

where gs represents growing season and ε represents residual error. A linear regression of the residuals (ε) from fitting the ANOVA model described above was accomplished, for which neither the intercept (t = -0.71; df = 1; P = 0.48) nor slope (t = 1.65; df = 1; P = 0.10) were significantly different from zero. In addition, there was no visual evidence of a trend in the variance of the residuals with variation in the square root of number of aphids per 12 leaves (Fig. S1). Therefore, we considered the ANOVA model to be appropriate for these data.

CANOCO® version 5.1 was used to statistically model relative aphid density and parasitism (number of parasitoids of each species emerging per sentinel plant) in canola fields to landscape variables. Partial redundancy analysis (partial RDA) was calculated for this purpose, where season (autumn and spring) and growing season (2016, 2017, and 2018) were included as covariables the effects of which on parasitism by species and landscape variables were removed. Although effects of season and growing season on aphids and parasitoids may be interesting in their own right, they were not the focus of our study, and their effects

were removed prior to forward step-wise selection of landscape variables. In CANOCO the partial RDA is calculated from the residuals derived from modeling effects of the covariables season and growing season (Šmilauer and Lepš 2014). Partial RDA was used because the length of the longest gradient in the landscape data was 1.56, which is less than the rule of thumb of 4.0 when a unimodal model such as correspondence analysis would be more appropriate for analysis (Šmilauer and Lepš 2014). The gradient length of 1.56 indicates that relationships among variables are approximately linear. Thus, the partial RDA modeled the relationships within and among both sets of variables, relative aphid density and number of *D. rapae* and *A. nigritus* per sentinel plant on one hand and landscape variables on the other. The step-wise forward selection procedure in CANOCO was used to select of landscape variables to include in the model. The forward step-wise inclusion method in Canoco provides accurate type 1 error rates and acceptable statistical power (Legendre et al. 2011). Landscape variables were entered sequentially from the most influential (greatest explained variance) to successively less influential based on contribution to explained variance, until the percentage of explained variation for inclusion of the next most influential variable was<10%. Monte Carlo resampling with 500 iterations was used to calculate a pseudo-F (F_p) statistic to test the significance of the partial RDA (Šmilauer and Lepš 2014).

3. Results

3.1. Patterns of aphid and parasitoid occurrence

Relative aphid density in canola fields varied markedly among growing seasons and between autumn and spring (Table 1). In each growing season relative aphid density was greater in spring than autumn. Two parasitoids, *D. rapae* and *A. nigritus*, parasitized turnip aphids on sentinel plants stationed in canola fields. *Diaeretiella rapae* was abundant and consistently present in fields, whereas *A. nigritus* was present but occurred sporadically (Table 1). Parasitism by *D. rapae* was greater in spring than in autumn of each of the three growing seasons. There was no clear trend for *A. nigritus* abundance among growing seasons. *Diaretiella rapae* parasitism on sentinel plants in different growing seasons did not correspond closely to relative aphid density in canola fields (Table 1). Parasitism by *D. rapae* was greatest on sentinel plants in autumn and spring of the 2017 growing season while relative aphid density in fields was greater in 2016 than in 2017. On the otherhand parasitism was lowest in autumn and spring of the 2018 growing season when relative aphid density was also lowest (Table 1).

3.2. Effect of parasitoids on aphids in exclusion and partial exclusion cages

The mean number of aphids per cage (total for 12 sampled leaves per cage) was greater in complete exclusion than in partial exclusion cages in the 2016 and 2017 growing seasons (Table 2). The number of

Table 1
Mean number of parasitoids emerging per sentinel plant and mean number of aphids per 60 D-vac placements (5.13 m² area sampled per field) for 23 winter canola fields sampled in the 2016, 2017, and 2018 canola growing seasons.

Growing season	2016	2017	2018
Number of fields	7	8	8
	$\overline{x}(SE)$	$\overline{x}(SE)$	$\overline{x}(SE)$
Autumn			
Diaeretiella rapae	1.60 (1.36)	11.75 (7.35)	0
Aphelinus nigritus	0.80 (0.80)	0	0
Aphids	15.8 (4.57)	10.1 (2.73)	0.63 (0.36)
Spring			
Diaeretiella rapae	12.29 (5.69)	229.13 (69.45)	10.00 (10.00)
Aphelinus nigritus	0.86 (0.86)	0	0
Aphids	373.4 (170.9)	87.6 (60.52)	3.5 (0.74)

Table 2

Mean number of aphids and parasitoid mummies in complete and partial exclusion cages stationed in winter canola fields in the 2016 and 2017 canola growing seasons.

Cage type	2016	2017
Number of cages	30	40
	$\overline{x}(SE)$	$\overline{x}(SE)$
Coarse		
Aphids	639.2 (90.35)	98.6 (42.86)
Diaeretiella rapae	32.7 (9.70)	6.4 (1.61)
Aphelinus nigritus	0.30 (0.17)	0
Fine		
Aphids	1329.7 (170.62)	330.6 (134.65)
Diaeretiella rapae	4.39 (1.62)	1.20 (1.13)
Aphelinus nigritus	0	0

parasitoid mummies was greater in partial than in complete exclusion cages in both years (Table 2). ANOVA for square root number of aphids per cage differed significantly among partial and complete exclusion cages within fields and growing seasons (F = 2.46; df = 14, 110; P = 0.005) indicating that aphid number were greater in complete exclusion than in partial exclusion cages. Significant differences also occurred among fields nested within growing seasons (F = 8.98; df = 12, 110; P < 0.0001) and among growing seasons (F = 164.1; df = 1, 110; P < 0.0001).

3.3. Landscape effects on aphids and parasitoids

Partial RDA calculated using forward selection of landscape variables was significant (F_p = 4.0; P = 0.014). Three landscape variables were incorporated in the partial RDA in decreasing order of percentage of variance explained by addition of the variable conditioned on variables already in the model: % wetlands (% explained variance = 12.6), contagion (% explained variance = 7.0), and % summer crops ((% explained variance = 3.3). The covariables season and growing season accounted for 10.6 % of the variance in parasitism while landscape variables accounted for 23.0% of variance. There was shared variance of 4.1% between the covariables and landscape variables. The relatively small percentage of shared variance between covariables and landscape variables indicates among other things that field location, which is never random due to logistical constraints, had a small effect on interpretation of effects of landscape variables on species abundance.

The similar direction of vectors in Fig. 3 for parasitism by *D. rapae* and the proportion of wetlands indicates a positive association between the two variables. Parasitism by *D. rapae* was unrelated to abundance of summer crops (indicated by the nearly perpendicular vectors for the two variables) and negatively related to contagion (vectors pointing in opposite directions). *Aphelinus nigritus* abundance was weakly (short vector) but positively related to the abundance of summer crops but was unrelated to other landscape variables (Fig. 3). Relative aphid density was weakly positively related to contagion and negatively related to abundance of wetlands (Fig. 3). Relative aphid density was negatively related to parasitism by *D. rapae* on sentinel plants but was unrelated to parasitism by *A. nigritus* (Fig. 3).

4. Discussion

Relative aphid density in canola fields was greater in spring than autumn of each of the three growing seasons. The increase in relative aphid density from autumn to early spring was not surprising and may have resulted from a combination of asexual reproduction by aphids residing in canola fields during the typically mild Oklahoma winters and immigration from other habitats (Jones et al. 2007, Royer and Giles
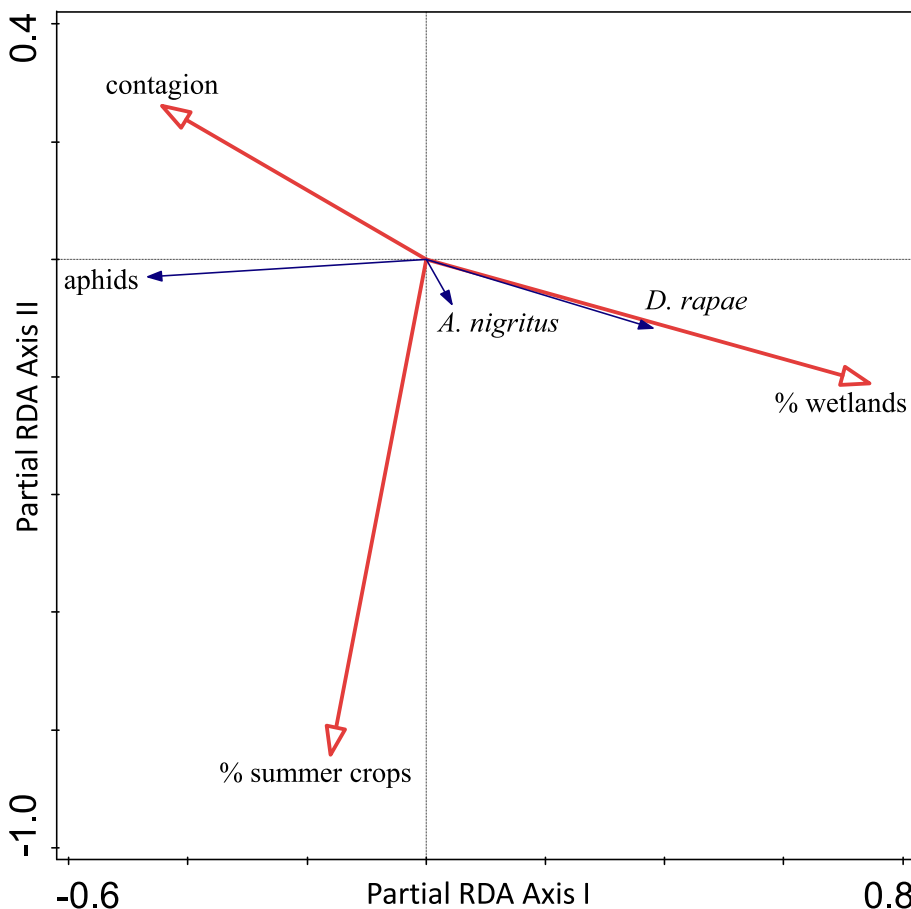

Fig. 3. Partial RDA ordination diagram for parasitoid species abundance in relation to landscape attributes within a radius of 1.5 km from the sampling location within each of canola fields. Variables were entered in the partial RDA sequentially by stepwise forward selection based on largest contribution to explained variance for the most influential remaining variable conditioned on variables previously included in the partial RDA, until the contribution to explained variance for inclusion of the next most influential variable was<10%. In the diagram pairs of vectors pointing in the same direction indicate a positive relationship between two variables, perpendicular vectors indicate that two variables are independent, and vectors pointing in opposite directions indicate a negative association between two variables.

2017). Parasitism by *Diaeretiella rapae* also was greater during March than November in each of the three growing seasons. Although foraging for hosts and reproduction by *D. rapae* in canola fields during winter has not been investigated in Oklahoma, it does occur for the parasitoid *L. testaceipes* in winter wheat (Jones et al. 2007, Giles et al. 2003). The marked increase in parasitism on sentinel plants from autumn to spring suggests that parasitoids survive and reproduce in canola fields during winter, probably supplemented by parasitism from *D. rapae* immigrating from other habitats in early spring (Giles et al. 2003, Royer and Giles 2017).

Two observations indicate that *D. rapae* reduced aphid infestations in Oklahoma canola fields. First, aphid numbers were lower and parasitoid numbers higher in partial exclusion cages compared to complete exclusion cages. Second, partial RDA demonstrated a negative relationship between parasitism by *D. rapae* on sentinel plants and relative aphid density in canola fields. Even though parasitism by *D. rapae* suppresses aphid infestations in canola fields it apparently is often not great enough to curtail economic injury, because many fields typically are treated with insecticide during spring to suppress aphids (Royer and Giles 2017).

Abundance of semi-natural habitats was associated with increased aphid parasitism by *D. rapae* in canola. However, not all semi-natural habitats were important. Landcover of wetlands was strongly associated with increased parasitism by *D. rapae* in canola, but land cover by grasslands and woodlands had no appreciable effect. Wetlands accounted for a small fraction of total land cover, ranging from 0 to 24.5 ha ($\overline{x}$ = 3.24 ha) from a total of 707 ha in a 1.5 km radius circle. In spite of the small proportion of total landcover wetlands were strongly associated with high aphid parasitism by *D. rapae*. The importance of wetlands to parasitism by *D. rapae* leads us to speculate that they maintain green foliage and host aphids and parasitoids at times during the year when other habitats are poor or unacceptable. For example, during the hot and droughty weather that frequents Oklahoma summers when many plants in upland habitats become dormant.

The importance of crop diversity for enhancing aphid biological control has been demonstrated in grain producing landscapes (Bosem Baillod et al. 2017, Redlich et al. 2018). Crop diversity in central Oklahoma mainly results from growing summer crops many of which have limited or no overlap in their growing season with that of winter canola and hence limited opportunity for dispersal of insects directly from summer crops to canola. Crop diversity in central Oklahoma was positively correlated to aphid parasitism by *L. testaceipes* in winter wheat fields (Elliott et al. 2018). However, we found no increase in parasitism by *D. rapae* in winter canola associated with landcover of summer crops. About 50% of the wheat planted in Oklahoma is dual purpose wheat planted for winter pasture for cattle and grain harvest and is planted about one month earlier (late August through September) than wheat planted for grain production only (Edwards et al. 2011). Due to earlier planting dual purpose wheat can bridge with summer crops, an opportunity that does not exist for winter canola which is typically planted in October (Royer and Giles 2017). Aphid hosts for *D. rapae* and *A. nigritus* occur in wheat and some summer crops. For example, *Schizaphis graminum* Rondoni and *Rhopalosiphum maidis* Fitch infest wheat and sorghum and are hosts for *D. rapae* and *A. nigritus* (Gilstrap et al. 1984, French et al. 2001), and *M. sorghi* infests sorghum and is a host for *A. nigritus* (Maxson et al. 2019). Our results suggest that opportunities are limited to enhance aphid biological control by parasitoids in Oklahoma canola fields by spatiotemporal manipulation of current cropping systems. And that manipulation of seminatural habitats and patch size and distribution may have more promise in that regard.

The absence of association of aphid parasitism by *D. rapae* in canola to landcover of wheat and grasslands, which dominate in total landcover in central Oklahoma (Table S3), indicates that these habitats are not significant sources of *D. rapae* to colonize canola fields, nor are they sinks for *D. rapae* emigrating from canola. Jessie (2017) demonstrated in the laboratory that *D. rapae* parasitized fewer aphids in microcosms

when *L. testaceipes* is present due to interspecific competition between the two species and speculated that parasitism by *D. rapae* in canola fields would be reduced when there was substantial landcover of wheat in close proximity to canola. However, Elliott et al. (2023) found that relative density of adult *D. rapae* in canola fields was 45 to 80 times greater than that of *L. testaceipes*, depending on growing season. Conversely, relative density of adult *D. rapae* compared to adult *L. testaceipes* is similarly low in wheat fields (Elliott et al. 2021). Host specialization occurs in *D. rapae* (Vaughn and Antolin 1998, Antolin et al. 2006, Derocles et al. 2016, Navasse et al. 2018, Ferguson et al. 2018, Derocles et al. 2020) and local adaptation to hosts may be a cause or consequence of habitat partitioning. With respect to winter crops grown in central Oklahoma as habitat for *D. rapae* and *L. testaceipes* there appears to be strong habitat partitioning and limited opportunity for interspecific competition.

The negative association of parasitism by *D. rapae* to contagion is notable. Correlations to contagion were negative and significant for patch density, SHDI, % wetland landcover, and % woodland landcover, and positively correlated to % wheat landcover (Table S2). Thus, contagion captures information contained in several landscape metrics that describe landscape heterogeneity inclusive of semi-natural lands. Contagion accounted for 7.0 % of explained variation in the partial RDA after accounting for variation explained by % wetlands. This observation highlights the fact that landscape metrics are contrived quantitative measures meant to capture aspects of heterogeneity that may or may not directly influence variables like species abundance. The influence on species abundance may sometimes be accounted for by less easily observable variables subsumed within the landscape metric.

In Germany Scheiner and Martin (2020) demonstrated that landscapes with diverse crop rotations exhibited greater and more stable aphid parasitism rates in cabbage, *Brassica oleracea* var. *capitata* L. They did not detect an effect of landcover of non-cultivated or semi-natural land on aphid parasitism by *D. rapae* in cabbage. In the state of Victoria, Australia Ward et al. (2021) observed that *D. rapae* was the most abundant aphid parasitoid in canola and was also present in wheat fields but was less abundant in wheat than other parasitoid species. *Diaeretiella rapae* was present in low numbers in field boundaries due to low abundance of acceptable hosts (Ward et al. 2021). This observation contrasts with a study of movement of natural enemies of aphid pests across habitat boundaries in New South Wales, Australia where *D. rapae* frequently moved from native vegetation into canola fields (Macfadyen and Muller 2013). Variation among studies in landscape attributes associated with aphid parasitism by *D. rapae* may be related to the broad range in ecological systems encompassed by the studies but may also be the consequence of methods used to describe landscape structure, which varied among studies and were coarse grained measurements in all studies. Many studies, including ours, fail to capture fine scale variation in landscape composition and configuration that may be important to plant-aphid-parasitoid dynamics, a well-known consequence of the scale dependence of ecological investigations and interpretation of underlying ecological processes (Turner et al. 2001). There is need for fine scale studies focused on the biological interactions that lead to observed broad scale patterns in studies such as ours. In Oklahoma for example, wetlands warrant detailed study of plant, aphid, and parasitoid associations because *D. rapae* apparently emigrate from wetlands to nearby canola fields.

Delayed colonization by natural enemies relative to pests in annual crops limits potential for successful biological control (Wiedenmann and Smith 1997, Wissinger 1997), and more specifically for biological control of aphids by *D. rapae* in brassica (Chua 1977, Neuville et al. 2016). Aphids and parasitoids that colonize canola in Oklahoma during autumn likely persist in the field through winter. Therefore, attributes of landscapes that facilitate early colonization and/or the magnitude of colonization in canola fields by parasitoids during autumn and early spring may have the greatest potential to improve aphid biological control in canola. The extent to which landscape structure can be manipulated to

elevate aphid biological control in canola to economically meaningful levels should be determined in order to progress to application in operational pest management programs. Application could be in the form guidelines for habitat management to increase biological control efficacy, guidelines for assessing potential for biological control based on knowledge of landscape structure, or both.

CRediT authorship contribution statement

Norman C. Elliott: Conceptualization, Methodology, Writing – original draft, Funding acquisition. **Kristopher L. Giles:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Kristen A. Baum:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Sarah D. Elzay:** Methodology, Writing – review & editing. **Georges F. Backoulou:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2023.105330.

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