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Flies Associated with Floral Canopies of the New Oilseed Crop, Pennycress, in the Midwestern U.S.A.

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Abstract

Flies are frequent visitors to flowers of many species of plants within the mustard family (Brassicaceae). They derive nutrition from these flowers, and some fly species are pollinators. Field pennycress (*Thlaspi arvense* L.) is a mustard species that is being developed as a new "cash cover crop," i.e., an autumn-sown cover crop whose oil-rich seeds can be harvested profitably in spring. Although pennycress is largely wind- and self-pollinated, its flowers also attract insect visitors. However, the extent of visitation to pennycress flowers by flies remains largely unknown, especially the identities of those flies. Thus, we examined flies associated with pennycress flowering canopies at five site-years in Illinois and Minnesota. The number of fly species averaged 16 per site-year. Hover flies (Syrphidae) were common visitors to pennycress flowering canopies, representing 24% of all Diptera collected. *Toxomerus marginatus* Say (margined calligrapher), whose larvae are aphid predators, was especially abundant within this family. However, the most common flies detected were *Delia* spp. (Anthomyiidae, root maggot flies), which averaged 51% of all flies collected. Adults of these flies are known pollinators, but their larvae also are pests that can damage seedlings of common summer crops. Although seedling damage to plants that are double- or relay-cropped (i.e., inter-seeded in spring) with pennycress has not been observed yet, close observation of this insect group and its effects may be needed if pennycress is widely sown in the future as a cash cover crop.

Keywords: Anthesis, Anthomyiidae, *Delia*, Diptera, pollinators, Syrphidae, *Thlaspi arvense*, *Toxomerus*, winter cover crop

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Resources provided by flowering cultivated crops, namely pollen and nectar, serve as important assets for many insect groups. Visits to flowers by such insects also may provide valuable pollination services for these crops. For crop pollination purposes, bees (Insecta: Hymenoptera: Anthophila) typically are considered the most important group of pollinators, while flies (Insecta: Diptera) often are thought to be the second most significant assemblage (Cook et al. 2020). Indeed, flies are especially important for pollination of plants belonging to the Brassicaceae (Jauker and Wolters 2008, Inouye et al. 2015, Orford et al. 2015, Phillips et al. 2018, Rader et al. 2019). This plant family includes the winter annual, field pennycress (*Thlaspi arvense* L.), of which there now are both wild lines (Best and McIntryre 1975) and new genetic lines that have been improved for their oilseed traits and ability to serve as winter cover crops to protect soil and the quality of soil water in cold agricultural regions, such as the Upper Midwest of the USA (Chopra et al. 2020, Marks et al. 2021). However, little information is available regarding the abundance and diversity of fly taxa that are attracted to either type of pennycress. Although pennycress flowers are largely wind-pollinated or self-pollinated, 9% of seeds can be attributed to insect pollination (Groeneveld and Klein 2014). Moreover, pennycress blooms extremely early in spring, and its flowers attract many early emerging insects (Eberle et al. 2015), which likely are drawn to its pollen and nectar (Thom et al. 2018).

Mulligan and Kevan (1973) listed three genera of hover flies (Diptera: Syrphidae) as occasional visitors to flowers of weedy pennycress in Ontario, Canada: *Eristalis* (three species), *Helophilus* (two species), and *Metasyrphus* (one species). Groeneveld and Klein (2014) reported insect visitors to spring-sown and summer-flowering pennycress in Germany, of which 27% were flies. Eberle et al. (2015) examined insect visitation to autumn-sown but spring-flowering pennycress plots at three site-years in Minnesota and South Dakota and found that 73% of all insects visiting pennycress flowers during this time were flies. At three site-years in Iowa and Minnesota, Forcella et al. (2021) found that flies represented 32, 61, and 84% of insects visiting spring flowers of autumn-sown pennycress. At these sites, insect visitation of pennycress flowers could be as high as 60 insects detected per minute by a single observer, but typical visitation rates were <10 insects min^{-1} (Eberle et al. 2015, Thom et al. 2018, Forcella et al 2021). Neither species, genera, nor families of Diptera were distinguished in these latter three studies. The high proportions of flies compared to other insect groups in these studies likely reflected the comparable and compatible phenologies of pennycress flowers and flies. That is, pennycress, if sown in autumn, is one of the first plants to flower in spring (as early as March in Iowa and April in Minnesota), and adult flies are often the first insects to appear in spring, as some flies over-winter as adults and larvae of others can develop (albeit slowly) at temperatures less than 5 °C (Hassell et al. 2017). For instance, adults of *Delia platura* Meigen (seedcorn maggots) emerge upon accumulation of as little as 200 growing degree-days (GDD, using 4 °C base temperature) after January 1st (Delahaut 2007). These flies are known to visit flowers and carry and deposit pollen on mustard type plants (Stavert et al. 2018) even on cold and cloudy days in alpine environments (e.g., Levesque and Burger 1982).

Each pennycress flower has four nectaries that secrete nectar and hold it in cup-shaped structures at the bases of the flower's four petals (Thomas et al. 2017). In wild-type pennycress lines nectar sugar production by flowers is rather low; only 12 kg of sugar ha^{-1} over the course of spring bloom (Eberle et al. 2015). However, nectary size and flower size are correlated, and both are under genetic control. Thus, breeding for larger flowers and greater nectar production is possible (Frels et al. 2019). Significant amounts of pollen, the primary source of protein and other nutrients for adult pollinators (and larvae of some pollinators), also are produced by pennycress: 38 kg ha^{-1} (Thom et al. 2018). Thus, current and future crops of oilseed pennycress, some recently commercialized (Marks et al. 2021), may represent important forage resources for many early-emerging and flower-visiting insects as well as critical winter-time components of relay- and double-cropping systems in regions such as the Upper Midwest of the USA (Cubins et al. 2019).

The purpose of the current study was to document the phenology, abundance and diversity of flies associated with flowering canopies of autumn-sown pennycress at four sites distributed from central and western Illinois to eastern and western Minnesota in the Midwestern USA. A primary goal of this work was to identify fly species that represent potential pollinators within this system as well as species that could benefit from early-season floral resources. Previous studies in this cropping system did not provide taxonomic resolution for the encountered Dipterans.

Materials and Methods

Seedbeds with loam soils were prepared with tillage and harrowing in small fields or large plots ($\geq$ 100 m^{-2}) in September or October at four research farms: Lexington IL (Illinois State University, 40.67° latitude, –88.78° longitude) in 2021, Macomb IL (Western Illinois University, 40.49°, –90.68°) in 2021, Rosemount MN (University of Minnesota, 44.71°, –93.07°) in 2020 and 2021, and Morris MN (USDA-ARS, 45.68°, –95.80°) in 2021. The proportions of cropped vs. non-cropped areas within a one-km radius of each site differed among the five site-years: 0.90, 0.75, 0.65, 0.70, and 0.60, respectively (Forcella et al. 2023). The non-cropped areas typically were comprised of woodlands, but a golf course abutted the Macomb site, a large drainage ditch flowed next to the Lexington site, and the Morris site was adjacent to a lake.

Pennycress seeds were sown in rows separated by 10 to 15 cm with a seeding rate of approximately 10 kg ha^{-1}. The genetic line sown at each site was 'MN106' (wild type) at Morris and Rosemount, 'B36/73' at Lexington, and 'tt8-t/ARV1' at Macomb (the latter two being genetic selections). Seedlings emerged at most sites within two weeks of sowing and formed rosettes that entered dormancy with the onset of winter. The exception was at the Lexington site, where many seedlings emerged in spring rather than autumn possibly due to dry soil conditions during and after sowing.

Flowering at each site was monitored at 7- to 10-day intervals during spring following autumn planting by photographing nine randomly situated 0.1 m^2 quadrats (three 0.25 m^2 quadrats at Lexington) and counting the number of open flowers in processed digital images. Monitoring commenced in April in IL and May in MN when flowers first were observed.

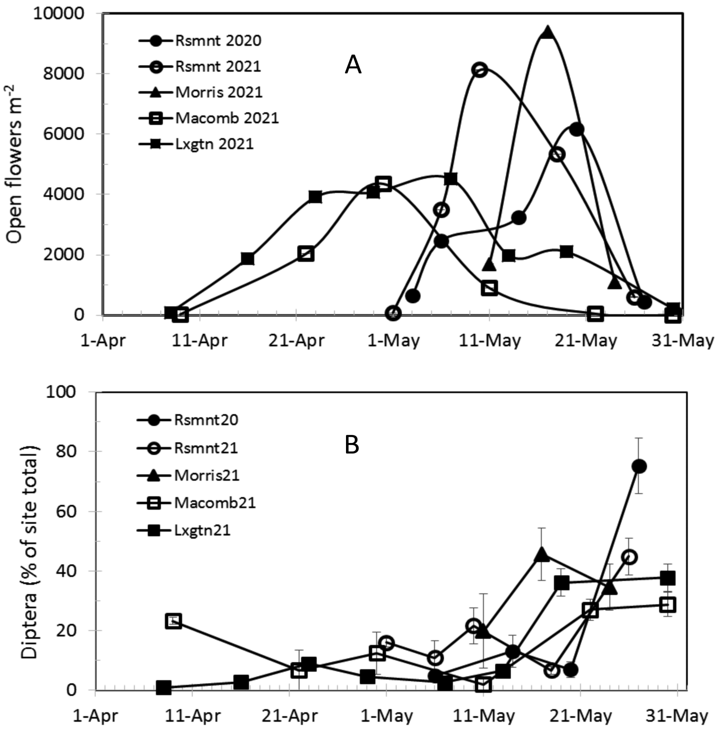

Figure 1. (A) Densities of open pennycress flowers, and (B) relative seasonal abundance of all dipterans (excluding midges, i.e., chironomids) associated with pennycress flowering canopies within each of five site-years: Rosemount, Minnesota, in 2020 and 2021 (Rsmnt20 and Rsmnt21); Morris, Minnesota, in 2021 (Morris21); Macomb, Illinois, in 2021 (Macomb21); and Lexington, Illinois, in 2021 (Lxgtn21). Vertical bars in B represent standard errors.

Insects were collected from pennycress sites on the same dates that flowering was monitored with the use of sweep nets, which can be very effective for capturing pollinating flies compared to pan traps (Berglund and Milberg 2019). Standard sweep nets with circular openings of 40 cm diameter were used. Sampling consisted of 50 sweeps (prostrate figure-8 design) skimming the tops of the flower canopies along three transects at each site; one sweep with each step of the person sampling. Transects were separated from one another by at least 10 m. At the end of each transect, contents of nets were placed in ice-filled coolers and subsequently (within 4 hours) transferred to freezers (–20 °C). Sweep netting occurred only on days without rain when air temperatures were >10 °C, and wind speeds were <15 km h^{-1}.

Subsequently, all captured insects from each transect were sorted by taxonomic order. Results for Hymenoptera are reported elsewhere (Forcella et al. 2023). All Dipteran specimens were dried, sorted, processed, identified and preserved at the Department of Entomology, University of Minnesota (St. Paul, MN). Identifications were made using available taxonomic resources (McAlpine et al. 1981, 1987; Skevington et al. 2019). Average daily air temperature data for each site were collected from the PRISM on-line source (https://prism.oregonstate.edu/explorer/) from the date of pennycress sowing to the end of anthesis. Cumulative heat units or growing degree days (GDD, base 4 °C) from sowing date to the end of May, as well as 1 January to the end of May, were calculated for each site-year. The latter calculation was for comparisons to literature values of fly phenology.

Results and Discussion

Pennycress flowers first appeared in April in Illinois and in May in Minnesota (Fig. 1A). The middle of the flowering periods at each site occurred upon accumulation of

Table 1. Cumulative precipitation from the dates of pennycress sowing in autumn through the end of May; cumulative growing degree days (GDD, base 4 °C) from pennycress sowing through the end of May; cumulative GDD from pennycress sowing to mid flowering dates; and dates of 200 GDD from 1 January for each of five site-years.

Site	Year	Precipitation (mm)	GDD from sowing to: End of May	GDD from sowing to: Mid flowering	Date of 200 GDD after 1 January
Rosemount	2020	630	746	532	13 May
Rosemount	2021	393	824	581	3 May
Morris	2021	251	743	562	15 May
Macomb	2021	648	837	541	13 April
Lexington	2021	507	1267	921 / 386 / 654*	10 April

*Spring emergence of pennycress seedlings was pronounced at Lexington; thus the three values represent GDD from (a) sowing to mid flowering, (b) 1 January through mid-flowering, and (c) the average of those two values.

an average (± S.E.) of 554 (± 11) $GDD_{4°C}$ after sowing (Table 1), except at Lexington, where ample spring emergence (≈67% of the population) of pennycress elicited an extended flowering season. High levels of seed dormancy and dry soils at sowing possibly explains low autumn emergence and abundant spring emergence at Lexington compared to the other sites, where spring emergence was scarce.

Overall, flies represented appreciable proportions of the total numbers of all insect taxa collected in sweep nets (Table 2). Most collections contained a high proportion of midges (Diptera: Chironomidae). Although abundantly represented in our samples, this group rarely visits flowers, and they are not considered important Dipteran pollinators. Consequently, midges were removed from our analyses to provide a more accurate view of potential flower visiting flies in this system. When midges were excluded from calculations, then non-midge flies, as flower visitors and potential pollinators, comprised 22 to 66% of the total potential insect pollinator populations when averaged across sampling dates within sites. With the inclusion of midges, the range for non-midge flies dropped to <1% to 48%. The lowest of these latter values was for the Morris, MN, site (adjacent to Swan Lake), where nearly three thousand aquatic midges were captured in the sweep nets; cumulatively averaging 954 (± 114) midges per transect. Midges have been excluded from all following results and discussion.

A total of 1018 fly specimens from 18 different families were collected (Table 3). The number of families represented per site-year averaged 8 (± 1.7). In total, 50 species were caught in sweep nets over both years during the flowering periods of pennycress, averaging 16 (± 4.2) per site-year. Anthomyiidae (root-maggot flies) and Syrphidae (hoverflies) were the two most common families, representing 51% (± 14.7) and 24% (± 10.5), respectively, of all collected flies per site-year (Fig. 2). The next most commonly represented family was the Chloropidae (frit or grass flies), which averaged 16% (± 9.5) of all flies collected per site-year. However, this latter family was only important in the two Illinois sites, having only minor representation in Minnesota. These three families combined averaged 91% (± 3.0) of the captured flies. Most other encountered families represented minor fractions of collected flies (Fig. 2; Table 3); rare exceptions are discussed below for individual site-years.

2020 Site-Year. In 2020, at the time open flowers were first recorded at the site in

Table 2. Percentages (± SE) of total insects (A) and total insects less midges (B) that were flies and considered potential flower visitors of pennycress at five sites during spring flowering periods of 2020 or 2021.

	Rosemount 2020	Rosemount 2021	Morris 2021	Macomb 2021	Lexington 2021
	%				
A	48 ± 6.1	26 ± 4.3	1 ± 0.1	24 ± 4.5	37 ± 0.6
B	61 ± 6.6	29 ± 4.6	22 ± 1.2	52 ± 3.8	66 ± 1.8

Table 3. Dipteran families (less Chironimidae), genera, species, and individuals caught in sweep nets during the flowering period of pennycress at five site-years. Site abbreviations: Rsmont = Rosemount, Minnesota; Lxngton = Lexington, Illinois; Mcomb = Macomb, Illinois.

Family	Genus	Species	Rsmont (2020)	Rsmont (2021)	Morris (2021)	Lxngton (2021)	Mcomb (2021)	Total
Anthomyiidae	*Delia*	*platura*	61	180	111	93	34	479
Asteiidae	*Asteia*	*beata*				1		1
Calliphoridae	Unknown	sp. 1		3				3
	Phormia	*regina*	2					2
Chloropidae	Unknown	sp. 1				4		4
	Unknown	sp. 2			1	2		3
	Unknown	sp. 3				1		1
	Unknown	sp. 4				1		1
	Unknown	sp. 5		1				1
	Unknown	sp. 6				5	1	6
	Unknown	sp. 7				3		3
	Unknown	sp. 8				2		2
	Elachiptera	sp. 1				1		1
	Hippelates	*nobilis*				1		1
	Liohippelates	sp. 1		1		2	52	55
	Thaumatomyia	*glabra*		1		83	2	86
Dolichopodidae	*Dolichopus*	sp. 1				2	8	10
Empediidae	*Rhamphomyia*	sp. 1		3				3
Lonchopteridae	*Lonchoptera*	*bifurcata*				31	2	33
Micropezidae	*Rainieria*	sp. 1	6					6
Muscidae	Unknown	sp. 1		1		1		2
	Unknown	sp. 2				3		3
	Unknown	sp. 3		1				1
	Unknown	sp. 4				3		3
	Eudasyphora	sp.	1					1
	Unknown	sp. 5		1				1
Phoridae	Phoridae	sp.1				1		1
Sarcophagidae	*Sarcophaga*	sp. 1				1		1
Scatophagidae	*Scatophaga*	*furcata*				8		8

Sciomyzidae	*Dictya*	sp. 1	1			2		3
	Pherbellia	*parallela*		1				1
Sepsidae	*Sepsis*	*punctum*				1	1	2
Syrphidae	*Eristalis*	*dimidiata*	1					1
	Eristalis	*arbustorum*	1					1
	Eupeodes	*americanus*	1	1		4		6
	Eupeodes	*volucris*	1					1
	Eurimyia	*stipata*			3			3
	Helophilus	*latifrons*			1			1
	Meliscaeva	*cincctella*				1		1
	Orthonevra	*pulchella*	1					1
	Platycheirus	*quadratus*	15			4		19
	Syritta	*pipiens*					2	2
	Sphaerophoria	*contigua*	1	1	1	2		5
	Sphaerophoria	*philanthus*		1				1
	Toxomerus	*geminatus*	2			3		5
	Toxomerus	*marginatus*	125	11	7	60	16	219
Tachinidae	Unknown	sp.	3					3
Tipulidae	*Nephrotoma*	*ferruginea*	1				2	3
	Symplecta	*cana*	8			1		9
Ulidiidae	*Chaetopsis*	*fulvifrons*		4		4	1	9
Totals			231	211	124	331	121	1018

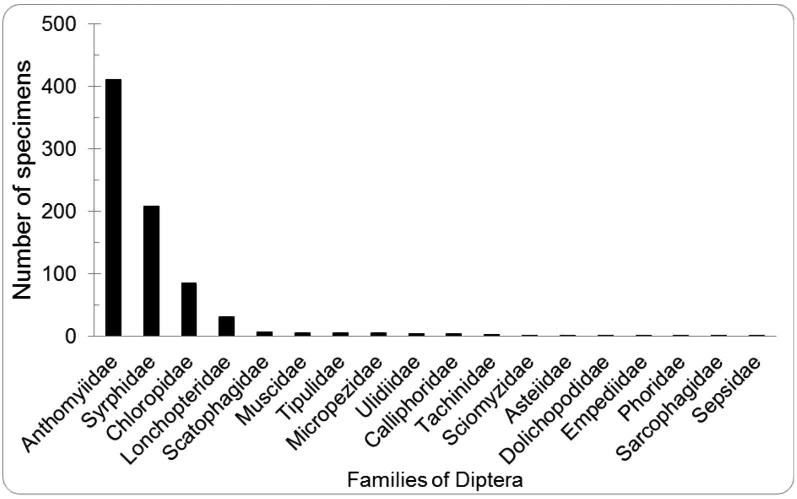

Figure 2. Total number of specimens across the spectrum of families collected from flowering pennycress canopies over five site-years in Illinois and Minnesota.

Rosemount, MN, flies were present (Fig. 1B), but their populations were very low until the end of May, when populations surged. Such surges likely represented adult emergence from locally metamorphosing larvae, but also could be due to adult migration from distant locales. Whatever the case, the most common family was Syrphidae, comprising 64% of all Diptera at this site. *Toxomerus marginatus* Say (margined calligrapher), which may be the most common syrphid fly in Minnesota (Telford 1939), was the dominant syrphid species, comprising 84% of all Syrphidae and 54% of all Diptera (Table 3). Adults of both *T. marginatus* and *T. geminatus* Say are common aphidophageous flies on flowers of mustard-type plants in North America (Harris-Cypher et al. 2023). The next most abundant syrphid at Rosemount in 2020 was *Platycheirus quadratus* Say (meadow sedge-sitter; 10% of Syrphidae and 6% of Diptera). Unlike most pollinators, *Platycheirus* spp. are flower flies tolerant of cloudy and even rainy weather conditions (Skevington et al. 2019). Seven other syrphid species were found in 2020, but together they represented just 5% and 3% of the total syrphid and dipteran populations, respectively (Table 3). Two of the *Eristalis* species collected in 2020 also were found on weedy pennycress in Ottawa, Canada (Mulligan and Kevan, 1973).

Delia spp. (Anthomyiidae: *Delia radicum* L., cabbage root fly; and *D. platura*, seed corn maggot or bean seed fly) was the next most common taxon at Rosemount in 2020, representing 26% of the total dipteran population. Adult specimens of *Delia* are difficult to distinguish at the species level; thus, they are hereafter grouped as *Delia spp*. Larvae of both species feed on plants in the Brassicaceae (Savage et al. 2016), but those of *D. platura* also feed on seeds and newly germinated seedlings of common crops (Dellinger and Day 2022) that can be double-cropped or relay-planted in spring into pennycress fields when the pennycress is bolting, i.e., when pennycress is forming flower-bearing stems (Cubins et al. 2019). *Delia platura* can produce multiple generations within a single growing season, with adults of the first generation emerging at 200 GDD after 1st January (Delahaut 2007). Such accumulation of heat units consistently occurred simultaneously with pennycress flowering (cf. Table 1 and Fig. 1A).

The remaining components (10%) of the May dipterans at the Rosemount site in 2020 included members of the following five families: Calliphoridae (*Phormia regina* Meigen), Micropezidae (*Rainieria* sp.), Muscidae (*Eudasyphora* sp.), Sciomyzidae (*Dictya* sp.), and Tipulidae (*Nephrotoma ferruginea* Fabricius and *Symplecta cana* Walker) (Table 3). At least four of the above species or genera include known flower visitors (Montgomery 1957, Maeda et al. 2015, Wiesenborn 2022, Marchiori 2023, Thomas et al. 2023,).

2021 Site-Years. Four sites were studied in spring of 2021. At Rosemount, flowering commenced in early May, peaked in mid-May, and concluded by the end of the month. The earlier flowering peak in 2021 than in 2020 possibly reflected the warmer and drier conditions in 2020–21 than in the previous growing season (Table 1). As in the prior year, more flies were caught in the later portion of the flowering season (Fig. 1B). Anthomyiids, all of which were *Delia spp*. (Table 3), accounted for 85% of the total fly population. Unlike spring of 2020, syrphids represented just 7% of all flies at Rosemount in 2021. The experimental area in 2021 was only about 500 m from that in 2020, but it was in the middle of a large tilled and cropped field. In contrast, the much smaller 2020 site was surrounded by wooded areas on three sides and a road verge on one side. All other flies together (six families) comprised 8% of all Diptera in 2021. These included Calliphoridae (unknown sp.), Chloropidae (*Liohippelates* sp., *Thaumatomyia glabra* Meigen, and unknown sp.), Empediidae (*Rhamphomyia* sp.), Muscidae (two unknown spp.), Sciomyzidae (*Pherbellia parallela* Walker), and Ulidiidae (*Chaetopsis fulvifrons* Macquart). Several of these taxa include known flower visitors (e.g., Kevan 1972, Larson et al. 2001, Ohler et al. 2016, Klepzig et al. 2022), but others such as *P. parallela* and *C. fulvifrons* do not, which suggests these latter species may have been merely in transit across pennycress plots when caught in sweep nets.

The site near Morris, MN, experienced a pronounced drought during the 2020–2021 growing season (Table 1), which shortened the flowering period of pennycress in 2021 to two weeks (Fig. 1A). Fly abundance over this short period of time mimicked pennycress flower densities, with most of the fly population being *Delia* (90%; Table 3). Syrphid flies (solely *T. marginatus*) represented 10% of all Diptera. Only one other fly family was detected in Morris in 2021 (Chloropidae), which was <1% of the total fly population.

At Lexington, IL, in 2021, the pennycress flowering season was extended greatly almost certainly because of combined autumn and spring seed germination and seedling emergence. Accordingly, greater numbers and diversity of flies were detected compared to other site-years. Fly populations were highest toward the end of the flowering season (Fig. 1). *Delia* was the most abundant genus (28% of all Diptera, and 100% of Anthomyiidae), but the most common family was the Chloropidae, which was represented by 11 species and accounted for 32% of all Diptera (Table 3). The dominant species within this family was a known flower visitor (Ohler et al. 2016), the frit fly, *T. glabra* (80% of Chloropidae, and 25% of all Diptera). Syrphidae comprised 22% of all flies in Lexington during 2021, again with *T. marginatus* being most common (81% of Syrphidae, and 18% of all Diptera). Additionally, one species in the Lonchopteridae (*Lonchoptera bifurcata* Fallen) was found. This insect is normally associated with stream margins and wetlands (Drake 2002) and is a recognized flower visitor (Thomsen and Sigsgaard 2019). It represented 9% of all Diptera. The pennycress field in Lexington was adjacent to an irrigation canal, which likely explains the presence of *L. bifurcata* in the samples.

Ten other families of flies were found in Lexington in 2021, representing 9% of the total flies captured. These families included Asteiidae (*Asteia beata* Aldrich), Dolichopodidae (*Dolichopus* sp.), Muscidae (three unknown spp.), Phoridae (unknown sp.), Sarcophagidae (*Sarcophaga furcata* Hardy, unknown sp.), Scathophagidae (*Scathophaga furcata* (Say)), Sciomyzidae (*Dictya* sp.), Sepsidae (*Sepsis punctum* Fabricius), Tipulidae (*Symplecta cana*), and Ulidiidae (*C. fulvifrons*). Many of these taxa are known anthophiles (Kevan 1972, Willmer 1982, Batra 1985, Larson et al. 2001, Ollerton et al. 2009).

At the site in Macomb, IL, pennycress flowering began in mid-April, peaked on about 1 May, and declined appreciably by mid-May (Fig. 1A), which likely reflected nearly complete autumn germination and emergence. As at the other sites, more flies were captured toward the end of pennycress flowering than earlier (Fig. 1B). When flowering commenced, the only flies detected were *Delia spp*. They represented 28% of all dipterans collected throughout the bloom period. However, the most commonly represented family was the Chloropidae, which accounted for 45% of all flies. This family was dominated (95%) by *Liohippelates* spp., which represented 43% of all flies at Macomb in 2021. Species within this genus, commonly known as eye gnats, typically are considered pests, but adults are known to visit flowers for nectar and pollen (Klepzig et al. 2022). Syrphids comprised only 15% of the total fly population, with *T. marginatus* dominating (89%) this family, but only accounting for 13% of total flies. Five other fly families (12% of all flies) were noted at Macomb in 2021. These were Dolichopodidae (*Dolichopus* sp.), Lonchopteridae (*L. bifurcata*), Sepsidae (*S. punctum*), Tipulidae (*Nephrotoma flavipennis* Morge), and Ulidiidae (*C. fulvifrons*). The composition of these minor groups was somewhat similar to those of other site-years.

These results for the spring Diptera assemblages associated with flowering of autumn-sown pennycress in the Midwestern USA (i.e., the Corn Belt) show domination by a small number of species, namely *T. marginatus* and *Delia spp.*, which are known to use floral resources and be effective pollinators. The collection was also rich in family and generic diversity representing a varied assortment of taxa whose abundances typically were low. Importantly, a clear trend was found for increased fly numbers as in-field flowering progressed. Thus, the results indicate that a broad array of flies may use flowers of pennycress if this crop eventually is grown across the widespread Midwestern agricultural environment.

While the majority of species collected in this study were recorded at low abundance, several key findings were clearly evident. First, many species known to use either nectar or pollen as a resource were found in this system. Pennycress provides valuable resources in the form of pollen and nectar in early spring when other floral resources are scarce (Thom et al. 2018). Syrphids, a group that is strongly associated with using floral resources and pollination services (Ssymank et al. 2008), were the most diverse group of flies found during this study. In particular, *T. marginatus* was abundant and present at all sites. While no other syrphid species was found at all sites, syrphids accounted for 7% to 64% of the species abundance across sites, averaging 24% (± 10.5). Additionally, several of the more commonly collected families contain taxa known to obtain nectar from a variety of floral resources. These groups include chloropids (Bohart et al. 1970, Klepzig et al. 2022), lonchopterids (Stalker 1956), scatophagids (Batra 1985), and anthomyiids (Finch 1971, Nilsson et al. 2011). This suggests that these taxa could be using flowering pennycress as nutritional resources.

The occurrence of these flies in this crop may lead to increased pollination services. Indeed, pollen transfer has been documented for the most commonly collected species in this study, *T. marginatus* (Chisausky et al. 2020) and *Delia spp*. (Stavert et al. 2018, Cook et al. 2020), and even for less common and physically small genera such as *Liohippelates* (Klepzig et al. 2022) or *Lonchoptera* (Stalker 1956). For comparison, pollen loads of *T. marginatus* are about one-fifth of those of *Apis mellifera* L., honey bees (Chisausky et al. 2020). Although pennycress does not require interactions with visiting flies for pollination, it does receive some benefit from insect visitors over wind pollination alone (Groeneveld and Klein 2014). Also, plants that require insect pollination surrounding agricultural fields are known to benefit from increased floral resources in the surrounding landscape (Blaauw and Isaacs 2014). Thus, the presence of these flower visiting Diptera at the studied sites further suggests the potential for beneficial services, which may include increased seed set following flower visitation, particularly for later-opening pennycress flowers.

The increase in fly numbers during flowering, across all sites, also indicates that many adult flies emerge simultaneously with the bloom period of pennycress. In a previous study from Iowa and Minnesota, fly visitation on flowering pennycress was correlated with sampling date and flower abundance (Forcella et al. 2021). The positive relationship with sampling date likely occurred because pupae of many fly species do not metamorphose into adults until after the peak of pennycress flowering. In the current study, adult flies at most sites were most abundant toward the end of the pennycress flowering period. Only at the Morris, MN, site was fly abundance correlated with flower density across sampling dates. Accordingly, flies may contribute only modestly to pollination of early-opening pennycress flowers at most sites, but later opening flowers likely experience greater visitation by pollen-laden flies, especially given the fact that up to 50 species of flies are associated with the flowers of pennycress. While we did not quantify the transport of pennycress pollen by these species, the nature of flower visitation and pollen transport by these groups (e.g., Chisausky et al. 2020) provides evidence for such activity to exist.

Beyond the potential for pollination services, the diversity and abundance of aphidophagous flies collected here further suggests that the provisioning of floral resources also may enhance regional biological control. Larval *T. glabra* (52% of Chloropidae, and 8% of all Diptera) feed on root aphids of crops such as bean and sugar beet (*Beta vulgaris* L.) (Yarkulov 2019) and, therefore, may have agronomic benefits other than potential pennycress pollination, especially in Illinois where this fly was abundant. The larvae of the more commonly collected genera such as *Toxomerus*, *Sphaerophoria*, *Platycheirus*, *Eupeodes*, and *Eristalis* all feed on soft-bodied insects such as aphids (Chisausky et al. 2020). Larvae of *T. marginatus*, in particular, are predators of pest aphids in soybean fields. However, recent research near the Rosemount study site indicated that larval populations of these flies in soybean were so low that little control of aphids occurred (Eckberg et al. 2015). Perhaps if adults of this fly have better access to early spring forage resources (e.g., pennycress pollen and nectar), more fly eggs, fly larvae, and aphid predation can be expected in relay-cropped soybean. Thus, even if *T. marginatus* is not an important pollinator of pennycress, its dietary health may be important for soybean that is relay-cropped with pennycress.

One unforeseen result was the degree to which flies of the genus *Delia* dominated the Diptera fauna collected during this study. The larvae of *D. platura* (corn seed maggot/bean seed fly) and *D. radicum* (cabbage root fly) are pests of plants in the Brassicaceae. However, their adults are also known pollinators of these same plants (Cook et al. 2020). Nectar of early-blooming plants is an important energy source for adults of these flies (Finch 1971, Nilsson et al. 2011), and likely contributed to their presence at all study sites. However, large populations of these flies were found only at three site-years: Rosemount 2020, Rosemount 2021, and Lexington 2021. Manure applications are known attractants for egg-laying by *D. platura* (Delahaut 2007). Soils at or adjacent to the research sites at Lexington and Rosemount had been amended with manure, but not those at Macomb or Morris, which may help to explain differences in *Delia* abundance across the site-years (Table 3).

Dry soil environments also are known to inhibit larval development of *Delia* (Hazzard 2016), which may have affected populations at Morris in 2021. In contrast, the very large populations of *Delia* observed in Lexington 2021 possibly can be explained by the very high number of cumulative growing degree days at that site-year (Table 1), nearly double that of other site-years, and this may have stimulated the high emergence rate of adult flies. Indeed, by late-flowering in Lexington (23 May), 600 GDD had accumulated after 1 January, which exactly matches the $GDD_{4\,°C}$ requirement for emergence of the second generation of adults of *D. platura* (Delahaut 2007). No other site surpassed 600 $GDD_{4\,°C}$ by the end of May.

Conclusions

The Midwestern fly assemblage associated with this new oilseed crop is diverse, and it offers at least three potentially positive outcomes: enhanced agroecosystem diversity, increased seed set of the crop, and biological control for pests of crops relay-planted into pennycress or double-cropped after pennycress seed harvest. However, a negative outcome also is possible. If pennycress is adopted extensively by growers as a new oilseed crop, close scrutiny of *Delia spp*. populations likely will be necessary. Although, to date, no significant damage by larvae of *Delia spp*. has been observed on pennycress plants (root crowns), or seeds and seedlings of crops relayed or double-cropped with pennycress, the high populations of this fly in fields of pennycress may increase the probability of pest adaptation to this new crop and cropping systems. Thus, close scrutiny of *Delia* populations will be necessary.

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