



Mutualistic and antagonistic trophic interactions in canola: The role of aphids in shaping pest and predator populations



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HIGHLIGHTS

- Complex food webs can influence when and where pests become problematic in cropland.
- Aphids attract mutualistic ants and a suite of predators; these interactions influence the entire arthropod community.
- Aphids, ants, and predator populations were positively correlated with one another.
- The presence of aphids and its associated predatory arthropod community were negatively associated with imported cabbageworms.
- Small aphid populations in cropland may be important for maintaining reduced levels of key crop pests.

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ABSTRACT

Aphids have important effects on the abundance and occurrence of tending ants, predators, and pests in agronomic systems, and DNA-based gut content analysis can aid in establishing predator–prey interactions. The purpose of this study was to determine how the presence of aphids, ants, and pest individuals interact within canola (syn. oilseed rape) fields. Using seasonal data from canola fields, the relationships among ants, aphids and pest individuals were determined, along with the use of PCR techniques in order to amplify aphid DNA and confirm food web links on predators who consume aphids. We determined that aphid presence positively influences the number of ants and predators in a community, and diminishing aphid populations over the growing season were associated with declines in both ants and predators. These reduced populations of predators and aphids may have provided the opportunity for a key pest, *Pieris rapae* to build populations as the season ensued. This research suggests that complex interactions among herbivores and shared predators contribute to pest outbreaks.

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1. Introduction

The green peach aphid (*Myzus persicae* [Sulzer]; Hemiptera: Aphididae) is a worldwide pest that occurs on a wide variety of crops (Desneux and Ramirez-Romero, 2009), including canola (syn. oilseed rape; *Brassica napus* L. Brassicales: Brassicaceae) (Farooq and Tasawar, 2008). Canola plants are most susceptible to damage by aphids during bud formation and through the late flowering stage (Berlandier, 2014). Large aphid infestations in early spring can cause wilting, abortion of flowers, and reduced pod setting in canola, which can result in yield losses (Berlandier, 2014). The increase in canola production in recent years in the

Midwestern states of North America has accompanied an increase of green peach aphid populations (Weiss et al., 2013). Like other aphids, green peach aphids feed on the phloem of their host plants (Mitter, 1958; Lundgren, 2009; Lach et al., 2010; Yao, 2014) that is rich in carbohydrates and poor in other nutrients and amino acids, resulting in the production of sugary honeydew (van Emden et al., 1969; Lach et al., 2010). This honeydew often acts as a food source for many ant species, resulting in the formation of mutualistic interactions.

In return for honeydew, tending ants often move aphids to new plant tissues (Hölldobler and Wilson, 1990; Gonzalez Hernandez et al., 1999; Finlayson et al., 2009), reduce debris buildup causing fewer disease outbreaks (Bach, 1991), and protect the aphids from potential predators (Way, 1963; Stadler and Dixon, 2008). Those aphid colonies that are tended by ants often see an increase in population numbers (Way, 1963; Cushman and Addicott, 1989; Del-Claro and Oliveira, 1993; Fischer and Shingleton, 2001;

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Dibble, 2009) due in part to reduced predation and enhanced feeding (Bristow, 1984; Del-Claro and Oliveira, 2000; Flatt and Weisser, 2000). These mutualistic interactions often have important effects on other species that are present within the shared system and food web.

The imported cabbageworm (*Pieris rapae* L.; Lepidoptera: Pieridae) oviposits and feeds only on those plant species that produce glucosinolates (Hopkins and van Loon, 2001), one of which is canola. Complex interactions of competing herbivores and predators present on potential host plants can alter when and where a female *P. rapae* lays her eggs. Aphid presence on potential host plants does not deter oviposition by these butterflies (Layman and Lundgren, in press), creating scenarios where the two herbivores share individual host plants. Since these two insects often occur on different parts of the plant (Harcourt, 1963) they have minimal contact with one another. Ants and other predators, however can affect both where eggs are laid, and overall survival of offspring. Some butterflies use visual cues to determine if predacious ants are present on potential host plants, and alter their oviposition preference accordingly (Sendoya et al., 2002). The presence of other predacious insects also alter egg laying behavior by changing chemical cues on plants and forcing females to search for enemy free space to ensure survival of their offspring (Gilbert, 1979; Holloway and Herbert, 1979; Price et al., 1980). Many ants are considered generalist predators (Hölldobler and Wilson, 1990) and often consume eggs and larvae of many insect species including those of *P. rapae* (Jones, 1987). Insect eggs are often considered as concentrated forms of nutrients due to their low water content (McNeil, 1971; Lundgren, 2009) making them high quality prey items for ants needed to maintain their colonies (Beattie, 1985). Predators can affect the maternal preference in combination with competitive herbivores by deterring females from laying eggs on those plants through the use of behavioral and pheromone interactions (Atsatt, 1981; Layman and Lundgren, in press).

A large, diverse variety of generalist predators inhabit *Brassica* crops, including canola, and contribute to reducing pests such as aphids and *P. rapae* larva numbers below economically damaging population levels (Gavolski et al., 2011). The main predators include various lady beetle species (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), and several families of spiders (Araneae). However, additional research on the bioinventory of insects in canola is warranted (Gavolski et al., 2011).

Previous laboratory research has shown that aphids in the presence of a predator can affect pest numbers (M.L.L. unpublished data). In order to determine if these interactions still occur in a field setting, we assessed the insect community of canola along with key predators that aid in reducing aphid population numbers. We also assessed the effects that ants, aphids, and *P. rapae* have on one another with collected population data, along with aphid predation that was occurring in the field. We believe that that aphid populations support ant and predator populations, and in turn these populations aid in suppressing *P. rapae* populations.

2. Materials and methods

2.1. Plants

Prior to the beginning of the experiment canola was planted in 10 blocks (24 × 6 m each) between 3.7 and 18 m apart. Canola seed (an open pollinated line without insecticide, Monsanto Company, St. Louis, MO) was planted at a density of 7.3 kg/ha in these no-till blocks within spring wheat residue with a fertilizer of 70-30-30-20 NPKS (Nitrogen-Phosphorous, Potassium, Sulfur), row spacing of 19 cm and a depth of 2 cm. Blocks were sprayed twice with the herbicide Clethodim (Select Max[®], Valent, Walnut

Creek, CA) at 840 g/ha, and blended with alkyl aryl polyoxylkane ether (Induce[®], Helena Chemical Company, Collierville, TN) at 350 g/ha, and once with the herbicide Clopyralid (Stinger[®], Dow AgroSciences, Indianapolis, IN) at 280 g/ha in order to remove grasses. Each block was then divided into 4 plots (3 × 6 m each) with a 3.6 m canola buffer in between each of the plots. Each plot was then randomly assigned a treatment, in which 6 plants were introduced into each of the 40 plots resulting in 240 plants overall. Experimental canola plants were also sprouted in peat pellets (Jiffy Products, Shippagan NB, Canada), and then transferred to soil mix (4:2:1 parts vermiculite: peat moss: field soil) at the first leaf stage. Half of the plants were grown in an aphid-free greenhouse, while the other half were grown in a greenhouse infested with *M. persicae*. Plants were watered daily, the temperature was 27 °C, and a photoperiod of 16:8 h light: dark (L: D) until the end of the second growth stage (≈12.7 cm tall).

2.2. Insects

M. persicae were reared from an established colony on canola plants within one of the greenhouses. Field ants were surveyed to determine nest density surrounding the experimental blocks by counting ant nests in the four neighboring field margins of alfalfa around the blocks, and also in each of the 10 canola blocks.

2.3. Experimental design

Three treatments were used to determine the role of ants and aphids on within-plant insect community dynamics on canola plants: ants and aphids, ants and no aphids, and no ants and no aphids. We initially planned to use a 2 × 2 factorial design that also included no ants and aphids, but we were unsuccessful in excluding ants from the plants when aphids were present, and so the treatments with and without ants with aphids were combined (following statistical analyses that showed no difference between these two treatments). Aphid-infested plants produced in the greenhouse ($n = 120$) were examined, and the number of aphids per plant was culled to 50 using a vacuum. Half of the un-infested plants ($n = 60$ each treatment) were subjected to the same ant exclusion procedure. Potted plants representing each of the three treatments were then placed in the canola field, plants in this field were 55 d old at the time of insertion of the potted plants on July 8th until August 4th. Each plot was randomly assigned a treatment, and six plants of the same treatment were placed randomly within each plot. A bamboo stake was inserted next to each pot and used to support the developing potted plant. Any plant material touching the pot, plant, or bamboo stake was removed, and the bottom 7.5 cm of the bamboo stake coated with tanglefoot in the ant-excluded treatment.

Starting at 24 h, insect communities on each of the focal canola plants were tabulated. These community assessments were conducted 1, 3, 8, 13, 17, 22, and 27 d after placement; until the surrounding field plants developed pods. During each observation, plants were non-destructively examined and all insects on the plant were collected and stored at −20 °C in 70% ethanol. Aphids in the aphid-infested treatment were allowed to remain on the plants. Following these whole plant counts, each plot was swept 25 times each with a 40.6 cm diameter insect net, and samples were stored at −20 °C in 70% ethanol. Arthropods collected in the whole plant and sweep samples were identified to species level to determine overall diversity within canola. Putative predatory species were advanced for gut content analysis of aphid predation. After the surrounding plants began setting pods, insect sampling ceased and all potted plants were removed from the field, and clipped at the soil level.

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