

Research Paper

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Impact of *Aphis pomi*-tending *Formica rufa* (Hymenoptera: Formicidae) on biological parameters of *Hippodamia variegata* (Coleoptera: Coccinellidae)

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Abstract

One of the key reasons for the poor performance of natural enemies of honeydew-producing insect pests is mutualism between ants and some aphid species. The findings demonstrated that red wood ant, *Formica rufa* Linnaeus (Hymenoptera: Formicidae) had a deleterious impact on different biological parameters of the lady beetle, *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae). *H. variegata* laid far fewer eggs in ant-tended aphid colonies, laying nearly 2.5 times more eggs in ant absence. Ants antennated and bit the lady beetle eggs, resulting in significantly low egg hatching of 66 per cent over 85 per cent in ant absent treatments. The presence of ants significantly reduced the development of all larval instars. The highest reduction was found in the fourth larval instar (31.33% reduction), and the lowest in the first larval instar (20% reduction). Later larval instars were more aggressively attacked by ants than earlier instars. The first and second larval instars stopped their feeding and movement in response to ant aggression. The third and fourth larval instars modified their mobility, resulting in increased ant aggression towards them. Adult lady beetles were shown to be more vulnerable to ant attacks than larvae. However, *H. variegata* adults demonstrated counterattacks in the form of diverse defensive reaction behaviours in response to *F. rufa* aggression.

Introduction

The most impactful aphid species on apples is *Aphis pomi* De Geer (Hemiptera: Aphididae). It was first described by De Geer from Sweden (Bhalla, 1972) and has since been recorded from all apple growing regions (Hamilton *et al.*, 1986). It is the most prevalent aphid species identified in Kashmir valley apple orchards (Khan, 2015). Yellowing of leaves, leaf withering, and retardation of plant growth are symptoms of green apple aphid infection (Opfer and McGrath, 2013). This insect pest infests apple plants all year and is a major problem for growers, causing significant losses in nurseries and orchards. *Aphis pomi* infestations can limit plant growth and increase lateral branch growth, particularly on young, non-bearing plants with high infestation levels on shoot tips. If green apple aphid populations are not controlled in time, large aphid colonies may form, resulting in lower production and harm to apple plants (Bouchard *et al.*, 1986). The species also secretes honeydew, which drips on the leaves and fruits and causes them to blacken. The afflicted trees and fruits become unsightly, lowering the market value of the crop. Honeydew is also a food source for some ants, which in turn defend aphid colonies on apple trees from predation (Khursheed *et al.*, 2021). Over many years, the extensive use of pesticides to control aphids has resulted in the development of aphid resistance to numerous kinds of insecticides (Ahmad and Akhtar, 2013). The best way to avoid the negative effects of chemical control, such as environmental contamination and insect resistance, is to employ biological control (Whitecomb and Bell, 1964; Dean and Sterling, 1992). A significant number of natural enemies are documented from various parts of the world against green apple aphid, *A. pomi* (Hagley and Allen, 1990). Lady beetles are the most important aphid predators among the numerous bio-agents (Hodek and Honek, 1996; Omkar and Parvez, 2000; Khan, 2009). Several species of aphidophagous lady beetles are frequently used for biocontrol of aphids in various crops and

cropping systems (Cabral *et al.*, 2009). *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) is a lady beetle that originated in the Palaearctic region, but is now reported to occur all over the world (Franzmann, 2002) and is one of the most commercialised predators utilised for the biological control of numerous aphid pest species in many economically significant crops (Krafsur *et al.*, 1996; Wheeler and Stoops, 1996; Kontodimas and Stathas, 2005; Mora *et al.*, 2020). It is a common coccinellid species in Kashmir Valley, found in a variety of agro-ecosystems (Khan *et al.*, 2007) and is particularly prevalent in aphid-infested apple orchards (Khurshid *et al.*, 2021).

Biological agents have been widely used in the past to efficiently manage ever-increasing aphid populations. However, new research has discovered that the roles of these biological agents are hampered by a variety of biotic and abiotic variables (Marchioro and Foerster, 2016). Among the biotic factors, ants are one of the most important biotic elements that affect the predatory capacity of lady beetles and defend aphid populations against predation by critical biological agents (Khurshid *et al.*, 2021). Ants also guard aphids from fungal illnesses (Nielsen *et al.*, 2010), and they relocate aphids to different feeding areas when a plant's quality is compromised (Majerus, 1994). Most investigations on the interactions of aphids, ants, and lady beetles show that ants rarely tolerate the presence of lady beetles in their environment (Lucas, 2005). The mutualisms between ants and some aphid species have received a lot of attention, and increases in aphid populations has been shown to be aided by ant protection from predators and parasitoids (Bishop and Bristow, 2003; Mooney, 2006). For instance, the survival of *Coccinella septempunctata* Linnaeus and *Hippodamia convergens* Guerin-Meneville larvae decrease by the fire ant, *Solenopsis invicta* Buren (Kaplan and Eubanks, 2002). Aphids often produce honeydew for ants in such mutualisms, and ants in exchange provide adversary free space for aphids (Minarro *et al.*, 2010). As a result, the biological control of aphids by lady beetles is compromised by the presence of ants, which fight and repel the lady beetles, lowering their efficacy (James *et al.*, 1999; Kaneko, 2002). Although, presence of *H. variegata* has been reported from various agro-ecosystems around the world and is thought to be a suitable biological control agent for aphids, no information is available on the impact of the red wood ant, *Formica rufa* Linnaeus (Hymenoptera: Formicidae), on the reproductive and developmental performance of *H. variegata*. *Formica rufa* is regularly found in *A. pomi*-infested apple orchards in Kashmir Valley. The purpose of this study was to determine the effect of *F. rufa* on some reproductive and developmental characteristics of *H. variegata*.

Materials and methods

The study was conducted at Pheromone Technologies Laboratory, Division of Entomology SKUAST-Kashmir. The studies were carried out in an ambient laboratory setting with temperature $27 \pm 2^\circ\text{C}$ and relative humidity $70 \pm 5\%$.

Conservation of stock cultures

Red wood ant, *Formica rufa* culture

An ant nest, which contained a queen and 200 to 300 workers, was collected from the SKUAST-Kashmir experimental fields. The ants were kept in a laboratory in a plastic container.

The container walls were painted with Sticky barrier/Fluon to prevent the ants from escaping. A potato plant (15 cm diameter pot) and an inverted peat pot (10 cm diameter) provided cover, with a moist sponge providing a steady supply of water. The nest was fed with 2–3 grams of cockroaches and other dead insects twice a week, as well as 0.5 g of granulated sugar and 2 g of chopped, boiled eggs as previously described by Finlayson *et al.* (2009).

Green apple aphid, *Aphis pomi* culture

The aphid culture was maintained on apple shoots in the laboratory. Growing apple shoots were gathered from insecticide free apple trees grown in the experimental orchards of the SKUAST-Shalimar Kashmir campus. These branches were placed in conical flasks filled with water and sealed with cotton to prevent aphids from falling into the water. The aphids were collected from unsprayed apple trees and released using a fine camel-hair brush on apple branches kept in conical flasks. These conical flasks were placed in the insect rearing cages. The apple shoots were replaced with fresh shoots as required, and the culture was maintained for future use.

Lady beetle, *Hippodamia variegata* culture

The *H. variegata* culture was started by collecting adults from the field and keeping them in plastic jars (20 cm length and 15 cm diameter) with an adequate supply of aphids. The culture was maintained pair by pair. Every 24 hours, the aphid supply was replenished. To aid oviposition, crumpled paper was placed in the rearing jars. The jars were checked daily, and eggs were retrieved and transferred using a fine camel hair-brush to clean Petri plates lined with wet filter paper, where they were allowed to hatch. In the trials, newly emerging larvae of *H. variegata* from the stock were employed. The culture was kept under laboratory conditions until the experiments were finished.

Effect of *Formica rufa* interaction on *Hippodamia variegata* fecundity

Ten pairs of newly emerged (two days old) *H. variegata* adults ($n = 10$) were isolated and kept in separate transparent plastic jars containing 1–2 branches of the host plant (apple) dipped in plastic vials containing water to keep them fresh. Two clear plastic boxes were joined together by transparent sticky cello tape and an open hole was made in the upper half of the adjoining wall. The purpose of joining the two plastic boxes was to prevent predators from being killed by ants and to offer enough space for *H. variegata* to lay eggs. A Fluon barrier was placed in a horizontal line just beneath the hole to prevent the ants from passing from one arena to the other and have their access limited to only to the bottom of the arenas, but lady beetles could still reach both arenas by flying over the barrier line. An ant was placed in one container, and the nymphs and adults of green apple aphid, *A. pomi* were released on the shoots in the container with an ant. Once the aphids had settled on the apple branches, pair of *H. variegata* from stock culture, pre-starved for five hours, was released in the container with an ant. To assist oviposition, crumpled paper was inserted in each rearing container. Thereby *H. variegata* were given the option of laying eggs in both arenas. One ant from the stock culture was released into

the aphid plastic container for two hours per day. The control treatment was maintained in a single jar without ants as described above. The eggs laid by each *H. variegata* pair in each container were collected and counted every day until the oviposition period was completed. The experiment was replicated ten times.

Effect of *Formica rufa* on hatching of *Hippodamia variegata* eggs

Ten freshly laid eggs of *H. variegata* were transferred onto filter paper, using a fine camel hair-brush ($n = 10$) and then randomly assigned to one of two treatments. For the first treatment, eggs were placed for 24 h in plastic Petri plates with sticky barrier coated edges containing one ant. Throughout a 5-min interval, the number of times ants palpated with their antennae and/or bit the eggs was counted. After that, the eggs were placed in a 9 cm Petri plate, and the number of larvae that emerged was counted. The second treatment was an ant-free control. In total, ten replicates were carried out. The percentage of viable eggs was finally calculated.

Effect of *Formica rufa* on larval development of *Hippodamia variegata*

Fifteen (one day old) *H. variegata* first instar larvae ($n = 15$) were placed on filter paper, and gently placed in the experimental arena with sufficient supply of green apple aphid. The aphids were maintained on leaf discs that were cut from the leaves of unsprayed healthy and young host plants (apple). Once the aphids and larvae settled on the leaf discs a single ant was given access to the arena for one hour each day. Over a 5-min period, the number of times the ants palpated and/or bit the larvae was counted. Observations were taken daily until the larvae reached their next development stage. The experiment was replicated ten times. The same experiments were then repeated with 15 *H. variegata* second, third, and fourth instar larvae (one day old). The control treatments were maintained ant-free. At the end of each experiment, the per cent reduction in larval development over control was also calculated. Three categories of contact were recorded, ranging from low to high aggressiveness following Godeau *et al.* (2009).

Aggression and response scores

Ant interaction with larvae were scored according to categories, whereby, 0 = Ants approach, walk, groom themselves, and ignore the larva; 1 = Ants use their antennae or labial mouthparts to palpate the larva; and 2 = Ants attempt to bite the larva while frequently straddling it.

Larvae response to ant interactions were scored according to four categories, whereby, 0 = no reaction; 1 = stopped movement; 2 = stopped feeding; and 3 = backed away/ran away.

Formica rufa interaction with *Hippodamia variegata* adults

An adult *H. variegata* was placed in an arena with two ants for one hour of foraging. Adult lady beetles and ants were observed interacting for 10 min. Adult ants and lady beetles interactions were separated into aggressive and reactive behavioural aspects. The number of times each behavioural element occurred (f = frequency) was recorded and used to calculate modified aggression and responses scores (Holway, *et al.*, 1998; Suarez, *et al.*, 1999;

Garnas, *et al.*, 2007). The aggression score of ants against adult lady beetles was calculated using the following formula (Finlayson *et al.*, 2009):

$$-1 \times fa + 1 \times fb + 2 \times fc + 3 \times fd + 4 \times fe + 5 \times ff \\ = \text{Aggression Score}$$

Where f is the frequency with which a specific behavioural element occurs, and the subscript letters denote the following behavioural elements: a = avoiding, b = prolonged antennation, c = opening mandibles, d = chasing, e = grasping/biting and f = trying to bite

Similarly, the lady beetle response score to ant aggression was calculated using the following formula (Finlayson *et al.*, 2009):

$$-1 \times fA + 1 \times fB + 2 \times fC + 3 \times fD + 4 \times fE + 5 \times fF + 6 \\ \times fG \\ = \text{Response Score}$$

Where f is the number of occurrences of a specific behavioural element and subscript letters denote the following behavioural elements: C = drawing in legs/antennae, D = preening, E = turning on back/flailing legs/fluttering wings, F = backing away/running away, G = flying away.

Statistical analysis

Lady beetle larvae counts and eggs in the two arenas of the ant presence and absence tests were compared using a Student's *t*-test. Before applying the test, the assumptions of normality was tested using a Shapiro Wilks test. Homogeneity of variance of the two treatments (ant presence and absence) was confirmed using Bartlette test (Snedecor and Cochran, 1983). The statistical tests were analysed using the R programme (Ihaka and Gentleman, 1996). The hatch rate of lady beetle eggs in control verses ant-treatments was compared using a Student's *t*-test. The frequency of each ant contact category, occurring with each lady beetle larval stage was compared using Least Significant Difference (LSD/CD) test.

Results

Effect of *Formica rufa* interaction on *Hippodamia variegata* fecundity

The ants frequently bite *H. variegata* adults. Concurrently, *H. variegata* laid significantly fewer eggs when ants were present. In the presence of ants, each female laid an average of 89.8 ± 9.0 eggs, compared to 231.6 ± 17.61 eggs without ants present ($t = 3.42$, $df = 18$, $P < 0.001$) (fig. 1).

Effect of *Formica rufa* on hatching of *Hippodamia variegata* eggs

On average 66% fewer *H. variegata* eggs hatched in the presence of *F. rufa* as compared to 85% in ant absence treatments. Out of every 10 eggs in the ant present treatment, an average 6.6 ± 0.73 eggs successfully hatched compared to 8.5 ± 0.72 eggs in the ant absent treatment. This difference was statistically significant ($t = 5.018$, $df = 18$, $P < 0.0001$) (Table 1).

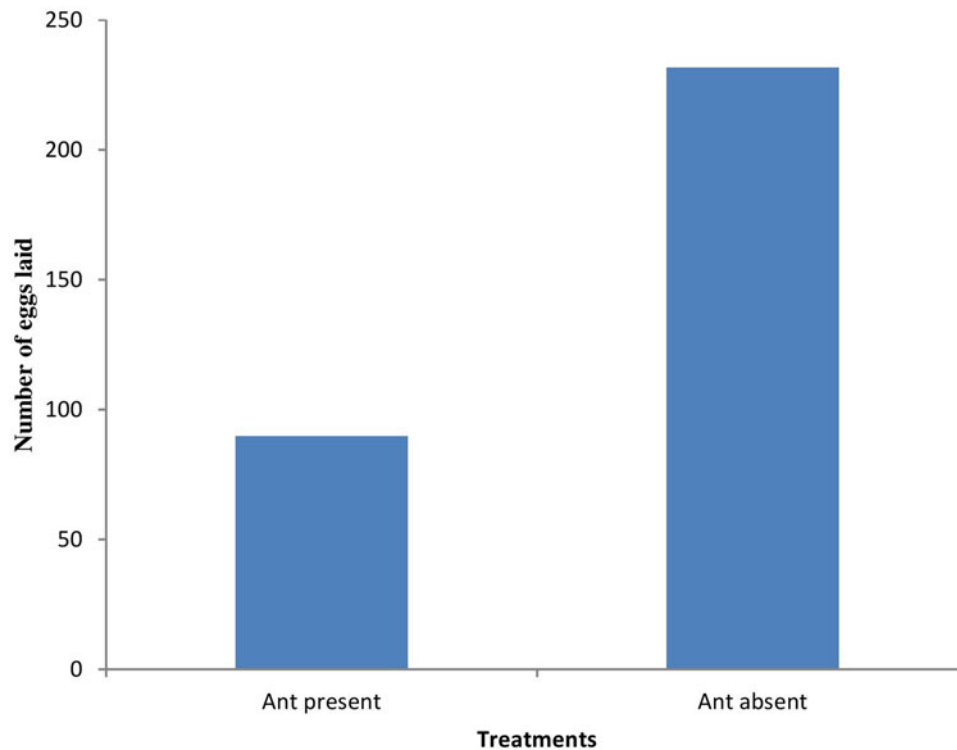


Figure 1. Effect of *Formica rufa* on fecundity of *Hippodamia variegata*. *H. variegata* females laid significantly less number of eggs due to the presence of *F. rufa* ($t = 3.42$, $df = 18$, $P < 0.001 \times t$ critical = 2.10).

Effect of *Formica rufa* on larval development of *Hippodamia variegata*

Ant presence resulted in significantly fewer *H. variegata* larvae surviving to each successive instar stage and ultimately to the pupal stage. An average of 9.9 ± 0.57 of the 15 1st instar larvae in the ant present treatment successfully grew into 2nd instar larvae, compared to 12.9 ± 0.63 without ants ($t = 5.018$, $df = 18$, $P < 0.001$). An average of 8.7 ± 0.64 of the remaining 2nd instar larvae in the ant present treatment successfully grew into 3rd instar larvae, compared to 12.2 ± 0.71 without ants ($t = 4.895$, $df = 18$, $P < 0.001$). An average of 7.7 ± 0.58 of the remaining 3rd instar larvae in the ant present treatment successfully grew into 4th instar larvae, compared to 11.8 ± 0.62 without ants ($t = 4.227$, $df = 18$, $P < 0.001$). An average of 6.9 ± 0.75 of the remaining 4th instar larvae in the ant present treatment successfully reached pupal stage, compared to 11.6 ± 0.82 without ants ($t = 3.473$, $df = 18$, $P <$

0.001). The maximum reduction in larval development was observed in 4th instar (31.33%) and minimum reduction in 1st instar larvae (20%) (Table 2).

Aggression and response scores

Ant interactions with *H. variegata* became more aggressive as development progressed. Ants ignored, bit and antennated the eggs of *H. variegata* an average of 2.0 ± 0.30 , 1.4 ± 0.30 and 0.9 ± 0.28 number of times per 5 minute, respectively. The ants ignored, bit and antennated first instar larvae an average of 1.50 ± 0.17 , 3.4 ± 0.65 and 3 ± 0.52 number of times per 5 min, respectively. In response to ant interactions, the first instar larvae temporarily stopped feeding and movement. The ants ignored, bit and antennated 2nd instar larvae on average 1.34 ± 0.15 , 4.7 ± 0.59 and 4.5 ± 0.54 number of times per 5 min, respectively. The 2nd instar larvae of *H. variegata* stopped feeding and limited their movement like the 1st instar larvae in response to the ant interactions. Ants only palpated and bit 3rd and 4th instar larvae. The mean number of times that ants bit and antennated the 3rd instar and 4th instar larvae during the 5 min period was 6.8 ± 0.51 and 7.1 ± 0.60 and 7.7 ± 0.37 and 8.8 ± 0.42 , respectively. The ants followed the larvae frequently, but the larvae quickly moved away from them to avoid interactions (Table 3).

Formica rufa interaction with *Hippodamia variegata* adults

When mature lady beetles and ants interacted, the ants did not accept the adult beetles near the aphid colonies and displayed all aggressive behaviours towards them, with biting being the most common behaviour. The ants aggression score towards lady beetles was calculated to be 55.65 ± 5.37 (fig. 2).

Table 1. Effect of *Formica rufa* on hatchability of *Hippodamia variegata* eggs

Treatment	Number of eggs taken per replication	Number of eggs hatched (Mean \pm SE)	Hatchability %
Ant present	10	6.6 \pm 0.73	66
Ant absent	10	8.5 \pm 0.72	85
Change observed		1.9	19
T test			

* = Significant
d.f. = 18, t stat = 5.0185, $P = 0.0001$ *, t critical = 2.100

Table 2. Effect of red wood ant, *Formica rufa* on development of different larval stages of *Hippodamia variegata*

Lifecycle stage	No. of larvae taken per replication	No. of larvae developed into next stage (Mean ± SE)		Reduction in larval development due to ant presence (%)	t value	df	P
		Ant present	Ant absent				
1st larval instar	15	9.9 ± 0.57	12.9 ± 0.63	20	5.018	18	<0.001
2nd larval instar	15	8.7 ± 0.64	12.2 ± 0.71	23.33	4.895	18	<0.001
3rd larval instar	15	7.7 ± 0.58	11.8 ± 0.62	27.33	4.227	18	<0.001
4th larval instar	15	6.9 ± 0.75	11.6 ± 0.82	31.33	3.473	18	<0.001

Similarly, *H. variegata* adults displayed a variety of reaction behaviours in response to ant interactions, including change in behaviour, changing movement, pulling in legs and antennae, turning on backs and flapping wings, running away/backing away, and flying away. In response to ant aggression, most lady beetle adults often choose to fly away from ant-tended aphid colonies to avoid ant interactions. The adult lady beetle response score was calculated to be 34.4 ± 2.62 (fig. 3).

Discussion

This study offers proof that *H. variegata* reproduction and survival are negatively impacted by the presence of *F. rufa* at aphid colonies. Several studies have shown that honeydew producing aphids attract ants to the plants they feed on. Therefore, it is evident that the mutualistic relationship between aphids and ants has a significant impact on the life cycles of arthropods in many types of crops (Eubanks, 2001; Kaplan and Eubanks, 2002). According to our findings, *H. variegata* fertility was much lower in females reared with *F. rufa* than in those who were not exposed to ant treatments. Many variables affect how many eggs are laid and how long the oviposition phase lasts. *Coccinella septempunctata* (Linnaeus) begins to lay eggs under field settings after the aphid population reaches a density of about 10 aphids per 1 m² of crop area (Honek, 1980). Similarly, *Adalia bipunctata* (Linnaeus) needed at least 10 aphids per 150 cm² in the lab to produce the maximum amount of oviposition (Hemptinne and

Dixon, 1991). The *H. variegata* given enough *A. pomi* in both treatments revealed that the presence of ants reduced adult aphid intake, which in turn reduced oviposition. Coccinellids are also supposed to boost their reproductive rates in response to non-prey meals, but they should avoid ovipositing in places where there is a lot of honeydew (Seagraves, 2009). According to a previous study, females with reduced prey intake produce fewer eggs due to their limited resource availability and many of these eggs do not hatch and are instead maintained in the ovariole (Agarwala *et al.*, 2008; Dehkordi *et al.*, 2013). Because of this, when the females are successful in locating aphids in large quantities, they are prepared to deposit eggs immediately (Evans, 2003). The egg-laying capacity of *H. variegata* might have been affected by ant aggression in our study in two ways: first, adult females may not have been receiving enough food, and second, the foraging area could have become inappropriate for egg-laying due to the presence of *F. rufa*. Based on our investigation, the site became unsuitable for *H. variegata* to lay eggs due to presence of ants. Previous studies also suggested that the availability of a suitable site has a substantial impact on reproductive success of *Aphidecta obliterate* (Linnaeus) and *A. bipunctata* (Cottrell and Yeargan, 1998; Schellhorn and Andow, 1999; Timms and Leather, 2007). The adult beetles' high dispersal abilities (Hodek, 1967) and their preference to remain and lay their eggs only in regions with high available aphid population density and suitable environments that are also hospitable to larval development and can support their new generations (Dixon, 1959).

Table 3. Behaviour and level of interaction of *Formica rufa* towards various life cycle stages of *Hippodamia variegata* during 5 min of interaction

Life cycle stage	Number of times ignored (Mean ± SE)	Number of times bitten (Mean ± SE)	Number of times antennated (Mean ± SE)	*Level of interaction	**Level of reaction of different larval instars
Egg	2.0 ± 0.30 ^a	1.4 ± 0.30 ^a	0.9 ± 0.28 ^a	0, 1, 2	–
1st larval instar	1.5 ± 0.17 ^{ab}	3.4 ± 0.65 ^b	3 ± 0.52 ^b	0, 1, 2	1, 2
2nd larval instar	1.34 ± 0.15 ^b	4.7 ± 0.60 ^b	4.5 ± 0.54 ^c	0, 1, 2	1, 2
3rd larval instar	0.0	6.8 ± 0.51 ^c	7.1 ± 0.60 ^d	1, 2	3
4th larval instar	0.0	7.7 ± 0.37 ^c	8.8 ± 0.42 ^e	1, 2	3
CD (P < 0.05)	0.58	1.47	1.32	–	–

Values sharing same letters in column are non-significant at $P = 0.05$.

*0 = Ants approached, walked, self groomed and ignored the eggs/larva, 1 = Ants palpated the larva with their antennae or their labial mouthparts, 2 = Ants bit the larva and simultaneously often straddled the larva.

**1 = stopped movement, 2 = stopped feeding and 3 = backed away/ran away.

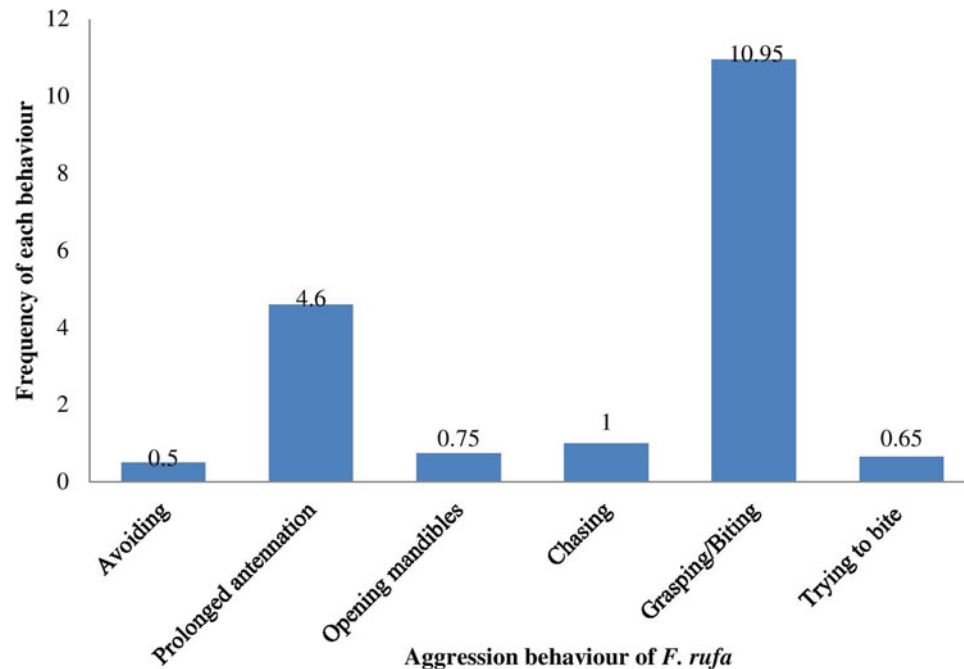


Figure 2. Frequency of different aggression behaviours of *Formica rufa* towards *Hippodamia variegata* adults with aggression Score of 55.65 ± 5.37 .

Our experiment showed that ants reduced the hatching of *H. variegata* eggs by 19% because they chewed and antennated the eggs. These results closely match those of Oliver *et al.* (2008), who found that ants damaged and chewed *A. bipunctata* eggs, which in turn lowered the hatching rate by 35%. The variance in the percentage of eggs hatching may result from the different species of lady beetle and ant. *Coccinella magnifica* (Redtenbacher) and *C. septempunctata* eggs are also frequently reported to be destroyed by *F. rufa* (Godeau *et al.*, 2009). Ants

didn't seem to eat the contents of the eggs, and the reason for this is not entirely clear however, the eggs contain poisonous alkaloids that may be the repulsive to the ants (Daloze *et al.*, 1995; Pasteels, 2007). Because coccinellid eggs have no physical defence, they are vulnerable to predation when laid on an exposed surface. So alternatively, ovipositing females have been seen covering their eggs with exuviae from aphid prey or depositing the eggs under prey scales (Pantuyukhov, 1968; Kawauchi, 1985).

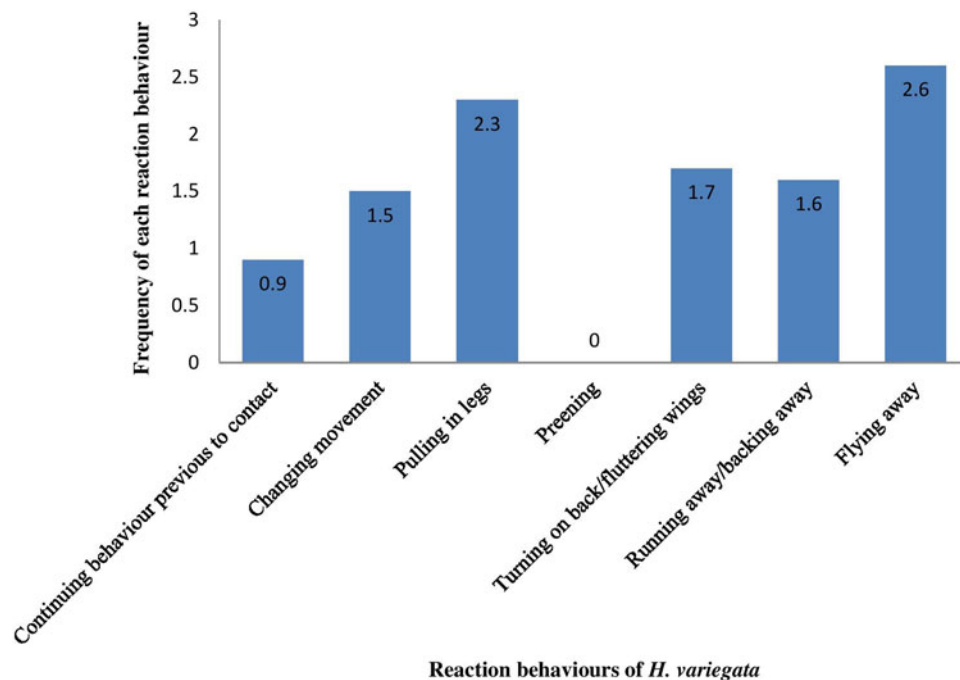


Figure 3. Frequency of different reaction behaviours of *Hippodamia variegata* adults in response of *Formica rufa* attack with reaction Score of 34.4 ± 2.62 .

The successful development of larvae was significantly reduced due to the presence of ants. Due to ants' increased aggression towards later instars, the biggest loss was observed in the 4th larval instar (31.33%), and the lowest in the first larval instar (20%). The increased aggression of ants toward later larval instars may be a result of their greater activity compared to earlier larval instars. According to our results, the first and second larval instars stopped their feeding and movement in response to ant attack, and the ants displayed low levels of aggression, whereas the third and fourth larval instars moved away from the aphids and the ants' aggression increased towards them. The less aggression of ants towards 1st and 2nd instar larvae could be attributed to their lower food requirement, which resulting in fewer contacts with ants and increased their survival chances. On the other hand, 3rd and 4th instar larvae with higher food requirements resulting in more contacts with ants, reducing their survival chances due to more ant aggression. These findings are quite similar to those of Huang *et al.* (2011), who reported that the presence of fire ants significantly reduces the survival of lady beetle larvae, with the later larval instars being more severely affected (reduction of 100%) than the earlier larval instars (reduction of 91.67%). It has been shown in our study that *F. rufa* greatly decreased the survival rate of all larval stages of *H. variegata*. All lady beetle larvae have strong self-defence mechanisms, including spines that can be utilised to fend off ant attacks for a brief amount of time (Richards, 1980; Sloggett, 1998). But, ants are still capable of killing larvae with such defences (Cheng *et al.*, 2015). The adult lady beetle is typically attacked by ants on their exposed parts, however they are externally protected by a strong sclerotised cuticle that pulls their legs, inward to avoid being stung and bitten by aggressive ants (Huang *et al.*, 2011). In our results, *H. variegata* exhibited various defensive behaviours included continued behaviour previous to contact, changed movement, pulled legs, turned on back/flattered wings, ran and flew away in response to *F. rufa* aggression. Our findings closely align with those of Finlayson *et al.* (2009), who found that different lady beetle species exhibit varying defensive strategies in response to ant aggression, with greater aggression ratings in species that appear to have more exposed morphology that ants may grasp. In addition to having elytra wings that appear to be an effective defence against ant attack (Jiggins *et al.*, 1993; Hodek and Honek, 1996; Volkl and Vohland, 1996), beetles can also exhibit behaviours that increase their survival against ant attacked. For example numerous beetle species pull their legs in close their body, and the level of ant aggression received is dependent upon beetle's broader camouflage in the immediate environment. A greater need for a lady beetle to feed increases the chance that a beetle will interact with ants and be attacked (Yasuda and Kimura, 2001; Rosenheim *et al.*, 2004; Takizawa and Yasuda, 2006).

Therefore, the food requirement of lady beetles would be one of the important factors in understanding the strength of interactions between lady beetles and ants mutualistic with aphids. In terms of the mutualism between the green apple aphid and red wood ant, the findings of this study confirm that the lady beetle, *H. variegata*, is a non myrmecophilous coccinellid with this aggressive ant species (*F. rufa*), but it could be myrmecophilous with non-aggressive ant species. A small number of coccinellids are myrmecophilous, and these species frequently reside near ant nests, in contrast to the majority of non-myrmecophilous coccinellids, which only consume ant-tended homopterans when untended homopterans are scared (Sloggett and Majerus, 2000). The differences in the size, aggressiveness and density of tending

ants and size, behaviour and defensive capabilities of coccinellids, undoubtedly affect the reproductive outcome of lady beetles.

Conclusion

We have determined that *F. rufa*, an aphid tending ant, negatively affects the reproductive characteristics of *H. variegata*. *H. variegata* is a crucial bio-control agent of multiple harmful insect pests, including aphids. However, because ants defend the aphids, which reduces the survival and reproductive capacity of *H. variegata*, the biological control capacity of this beetle is lowered by the presence of such ants. Therefore, if present, *F. rufa* must be managed prior to the release of *H. variegata* in order to successfully control *A. pomi*. In light of the consequences on mutualists and natural enemies, our research increased understanding of the ecological effects of the ant-hemipteran mutualism.

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