

## Preferences of the common garden ant (*Lasius niger*) in choice of aphid mutualist

Possibilities to increase biological control of the rosy apple aphid (*Dysaphis plantaginea*)

Preferenser hos svartmyra (*Lasius niger*) vid val av bladlusmutualist.  
En möjlighet att öka biologisk bekämpning av röd äppelbladlus (*Dysaphis plantaginea*)

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# Preface

When I first approached Mario for this thesis I thought that this was going to be a great deal of hard work, and time put in to the outcome, that is this paper. I turned out to be right. But I have never before learned so much about so little. And for that I am glad.

I would like to extend my gratitude and thanks to Mario Porcel and Joakim Pålsson at the institution of Plant Protection Biology at SLU-Alnarp for all the help and patience. I would also like to thanks the remainder of the institution of Plant Protection for having me along on this project. Other acknowledgements are extended to Gustaf Torpel for technical support and help, to my brother Johan Danielsson for tips and pointers on writing and language, and the personnel at the garden-lab at SLU-Alnarp for lending out tools and helping me out when needed.

Olle Danielsson

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# Abstract

The aim of this study is to acquire knowledge about the preferences regarding aphids of the ant *Lasius niger* (common garden ant), commonly found attending aphids and protecting them against their natural enemies in apple orchards around the world. By knowing this, it is hypothesized that another plant can be brought into an orchard carrying another aphid, more suitable as an ant-mutualist, to compete with the pest aphid present on site. The aphid *Dysaphis plantaginea* (rosy apple aphid), one of the key pests in apple orchards, was compared with *Aphis fabae* (black bean aphid), a common aphid that has been widely studied. This was done in an experiment involving multiple elements, including three arthropod organisms, apple trees and bean plants, in a semi-lab environment, to mimic real orchard-conditions. The result of the experiment was in undisputed favour of *Aphis fabae*, showing a significantly higher attention-rate by ants, and also a growing attention over time in comparison with the attention of *D. plantaginea* that did not increase. In conclusion, it can be stated that *A. fabae* is a better ant-mutualist and that the addition of this aphid could very well amplify the effects of biological control of *D. plantaginea* by natural enemies in orchards.

# Sammandrag

Målet med den här studien är att skaffa kunskap om myran *Lasius niger*s preferenser vad gäller bladlöss. *Lasius niger* eller svartmyran återfinns vanligtvis i odlingar över hela världen, där den lever i mutualistiska förhållanden med bladlöss och skyddar dem från sina naturliga fiender. Om man vet denna myrans preferenser är det föreslaget att en annan växt, infekterad med bladlöss som är bättre anpassade som mutualister till myran, skulle kunna introduceras i en odling. Detta för att konkurrera med redan befintliga bladlöss. Bladlusen *Dysaphis plantaginea* eller röd äppelbladlus, som är den största skadegöraren i äppelodlingar ställdes emot *Aphis fabae* (svart bönbladlus), en bladlus med en stor rad värdväxter, även den vanligt förekommande och vida studerad tidigare. Experimentet involverade tre insektsarter, äppelträd och bönplantor i en semi-labbmiljö för att på bästa sätt efterlikna förhållanden i en äppelodling. Resultatet av experimentet visade en otvivelaktig fördel för *A. fabae*, som uppvisade ett signifikant högre utbyte med myrorna i form av omhändertagande. Detta vårdande och omhändertagande av bladlöss från myrornas sida växte även signifikant med tiden i jämförelse med den uppmärksamhet som ägnades *D. plantaginea* som inte ökade. Sammanfattningsvis kan det konstateras att *A. fabae* är en bättre anpassad myr-mutualist och införandet av denna bladlus skulle kunna öka effekten av den biologiska kontroll av *D. plantaginea* i odlingar som dess naturliga fiender utgör.



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# 1 Introduction

## 1. 1 The driving forces or, Why we do what we do?

In this beginning section of my thesis I would like to try to explain roughly and rather briefly what my incentive has been in choice of subject. This is meant to give no in-depth description of the concepts I am about to portray, but rather to give the reader an idea of how I have gotten to where I am. And what I feel is important to understand for the reasons of doing this experiment.

## 1. 2 Ecosystem services

In the book *Nature's services: societal dependencies on natural ecosystems* (Daily 1997) the author discusses the need for functioning ecosystems to fuel a world habitable by mankind. Somewhat mockingly she describes a culture of seeing ecosystems as a subdivision of economy.

In light of that, the Millennium Ecosystem Assessment (MA) was initiated by former secretary-general of the United Nations (UN), Kofi Annan in 2000. This assessment is an anthropocentric evaluation of ecosystems, focusing on human health and benefits derived straight from the ecosystems around us. The assessment helped popularize the now commonly used term “eco system service”, which means just that, what the ecosystems around us can provide for us. This has become a way to quantify in money how much the nature is worth to us.

Four different services are mentioned in the MA, supporting, provisioning, regulating and cultural services. These entail for example, provisioning of food, fresh water, raw materials and bio-regulation, obvious needs to sustain human life on the planet. Above all the MA emphasized the risks and dangers in loss of ecosystems and biodiversity, (also in Mack et al. 2000) resulting in extension of loss of services, that may very well endanger life on earth. In chapter twenty-six of the MA, (Cassman, wood et al. 2005) they move closer to the fact that land-conversion into farmland is a major human-driven force that homogenizes ecosystems, and thereby damages or destroys them. However they also emphasize that an improvement of farming practises can conserve the biodiversity we risk to loose.

### 1. 3 Functional (agro)biodiversity

Altieri and Nicholls (2004) state in their book *Biodiversity and pest management in agroecosystems* that “clearly, the fates of agriculture and biodiversity are intertwined”. How this connects the biodiversity of farmland, or agroecosystems with the ecosystem services of the MA is what Altieri (1993) calls functional biodiversity, defined as “the biotic components that stimulate the ecological processes driving the agroecosystem and that provide the ecosystem services.” Functional biodiversity consists of functional groups (Moonen and Bàrberi 2008) and these groups comprise habitats or species present in the agroecosystem, as the name suggests, that have a function. Again for human benefit. So there is a clear distinction between functional biodiversity of farmland, that is used to keep up a function or provide a service, and biodiversity of farmland for conservation of species or genes for the sole purpose of keeping them alive. To clarify this, Moonen and Bàrberi state that the functional groups do not necessarily have to do with diversity what so ever, but can rather consist of monocultural elements, such as a single beneficial plant that is introduced into a system for a purpose.

Gurr, Wratten, and Luna (2003) explored the possibility of using functional biodiversity as pest management. In their review they discuss many different ways of implementing the changes, by 'relaxing' a monoculture, meaning to grow many varieties of a single crop, or adding another element of vegetation into the field (by letting weeds grow in patches that are managed and controlled, then harvested at different times and letting the residing predators migrate from one patch to another). This allows predatory insects to survive and thrive in a habitat that protects them and provides them with food and shelter as they also control pests in the cultivated surrounding.

### 1. 4 Habitat management

This is where we approach the term of habitat management. To apply these ideas of functional biodiversity one needs to arrange the existent agroecosystems devoid of previous non-crop elements. The simple explanation would be the creation of a habitat for a beneficial arthropod in order to supply a service, pest control for example. This is habitat management. An example of this is the reduction of aphid pests that was shown in orchards as a result of sheer diversity of adjacent plants to the crop (Lavandero et al. 2011). Also, by diversifying, or adding a functional group to the orchard (Walton and Isaacs 2011, Gontijo, Beers, and Snyder 2013, Song et al. 2013, Marko et al. 2013), commonly a flower strip to attract predators and parasitoids to establish and forage there.

## 1. 5 Pesticides and services

Another touchy, yet important aspect of the motives of this thesis is the reduction of pesticide use. Even if not scientifically always proven dangerous, pesticides is a subject of the general public not wanting them present in food or amongst their homes, due to the belief it will, in some way, affect them. In the year 2000 it was estimated that twenty-six million people were poisoned by pesticides each year (Paoletti and Pimentel 2000). The same could be said, but surely vastly greater in numbers, of insects and other living organisms in our agroecosystems. Chemicals pesticides have been shown to affect earth-worms (Liu et al. 2015, Wang et al. 2015), bumble-bees (Cutler and Scott-Dupree 2014), (Thompson et al. 2015) and bees, both wild solitary bees and domesticated ones, (Sandrock, Tanadini, Pettis, et al. 2014, Sandrock, Tanadini, Tanadini, et al. 2014, Tome et al. 2015). These are a very select number of articles that have described the effect of insecticides on some of our main beneficial insects when it comes to eco-system services. Some of the works show a 100% fatality of insects, yet others show only a small difference in lethality, with no significant effect, they do express the complexity of this that should be taken as a sign of caution for the future.

Even though from December 2013 the use of neonicotinoid-pesticides in Sweden and Europe has been prohibited (Swedish Chemicals Agency 2014), there are exceptions where farmers can spray both green-houses and fields (EU 2013). The prohibition only concerns crops “interesting to bees and other pollinators” (Swedish Chemicals Agency 2014), leaving out crops that are not pollinated by insects or crops that are harvested before bloom. Therefore these insecticides could still pose a threat to insects as residues in the soil as with the example of earth-worms stated above.

## 1. 6 The focus

The objective of this thesis is the functions of the addition of otherwise non-existent plant material into an orchard. Through this we hope to alter the focus of ants occurring in orchards and helping the natural enemies to suppress aphid pests. This would hopefully give an alternative to chemical pest-control.

## 2 Background

In this chapter the background of the experiment itself will be described, the elements that are used in the study and the role they play.

### 2. 1 Aphids

*Dysaphis plantaginea* Pass. (rosy apple aphid or RAA) is considered one of the major pests of apple-growing (Hill 1987, Blommers 1994, Arnaoudov and Kutinkova 2006). By feeding on the leaf underside or on young shoots the aphid damages shoots and fruit, causing them to not develop as normal and/or malforming them. This renders the apples affected unmarketable (Blommers 1994, Sandskär 2003, Arnaoudov and Kutinkova 2006) even with small colonies. The aphids can potentially damage the tree for coming seasons, causing previously infested branches not to set flowers the following year (Blommers, Helsen, and Vaal 2004). Obviously it is a pest that can have a disastrous effect, both in short term and in the long run. Therefore it is considered standard plan of action to control the rosy apple aphid in an early stage (Simon et al. 2011), before bloom (Pollini 2009), or even at a late stage in the autumn (Cross et al. 2007).



**Illustration 1:** Typical damage done by *Dysaphis plantaginea* to apple, curled leaves and shoots. Ants seen attending. Photo by Markus Hagenlocher.

A great deal of time and research has been put into controlling *D. plantaginea* as a pest

by means of biological control (Hemptinne et al. 1994, Wyss et al. 1999, Kehrli and Wyss 2001, Brown and Mathews 2007, Pålsson 2014), with the earliest publishing of biological control of the rosy apple aphid being in 1938 by P. Garman, the natural enemies and possibilities of arthropod control-agents have long been known.

## 2. 2 Ants and mutualism

It has been shown numerous times that the ant *Lasius niger* establishes a mutualistic relationships with aphids. The focus of this mutualism is a sugar-rich solution called honeydew that aphids excrete from their alimentary canal. This solution is attractive to ants as a high carbohydrate food source. The other end of the mutualistic relationship is that aphids receive protection from predators, and refuse management in form of ants disposing of aphid excrement, which has been shown to reduce disease in aphid colonies (Banks 1962, Way 1963, Buckley 1987, Wyss et al. 1999, Stewart-Jones et al. 2008, Miñarro, Fernández-Mata, and Medina 2010). Banks (1962), Buckley (1987) and Stewart-Jones (2008) also showed that the effect of not having ants present in the aphid colony would heavily decrease the amount of aphid individuals due to higher natural enemy predation.

Aphids compete for their ant-mutualists attention (Cushman and Addicott 1989, Fischer, Hoffmann, and Völkl 2001). A number of different aphid species and subspecies have been examined for quality of honeydew produced, (Völkl et al. 1999, Fischer, Völkl, and Hoffmann 2005) and from this it seems obvious that the aphid signature sugar, melezitose is a major attractant of ants (Völkl et al. 1999, Fischer, Völkl, and Hoffmann 2005, Detrain et al. 2010) Therefore it is hypothesized that a high melezitose producing aphid would correlate with a higher fitness as an ant mutualist. Fischer, Hoffman and Völkl (2001) observed ants abandoning aphids producing honeydew of lesser quality than others.

In this thesis, experiments will focus on testing two different species of aphid (*Dysaphis plantaginea* and *Aphis fabae scopoli*) against each other, in order to assess which one can compete for the ants attention, or, which aphid the ants choose in an apple orchard setting. In terms of attention it is hoped to draw focus to the one aphid, *A. fabae*, leaving the *D. plantaginea* unattended by ants, and therefore available as prey for predators and parasitoids.



**Illustration 2:** Ant attending *Aphis fabae*. Photo by Christoph Quintin

## 3 Materials and methods

### 3. 1 Insect material

#### 3. 1. 1 Ants

All ants in this experiment belonged to collected wild nests of *Lasius niger* L., the common black ant, or black garden ant. They all came from potted apple-trees where they nested from the previous season. The potted trees themselves were situated in apple-orchards around Skåne the year before. When placed there, the pots were un-colonized by ants. Thus, it is assumed that the ants originate from the different orchards as following, Orelund-, Helenelust-, Källagården-, Mejeriet-, Mandelmanns- and Dammstorp-orchard respectively.

The ants were collected by tilting the potted apple tree, pruning the roots that extrude from the pot in order to uproot them easier and lifting the lump of roots out of the pot to make sure the pot was colonized by the right ants. It was also checked for eggs and larvae as a sign of presence of a queen. The tree itself was cut using a hand-saw, and the remaining stem (approx. 10cm) was left as a handle for lifting. The root lumps with ants were then put in an ant-secured starving-box measuring  $576 \times 346 \times 407$  mm. They were kept there and starved, without food source or water, for 14 hours before each experimental block. During

the starvation-period the boxes with ants were kept in a greenhouse, also containing the remaining insects (aphids) and plant-material in order to keep everything under the same conditions.

### 3. 1. 2 Aphids

#### *Aphis fabae*

The black bean aphid (*Aphis fabae*, hereafter AF) originated from its winter host, *Euonymus europaeus* (European spindle) which was situated on campus (SLU, Alnarp). A single branch of the *E. europaeus* bush was collected and brought indoors on the 17<sup>th</sup> of April. The branch was left at room-temperature and the aphids were left to grow and multiply in a semi-protected area. Aphids were provided with a nearby bean-plant, *Vicia faba* (broad-bean, var. Gloria) so they could themselves choose to colonize it. The *V. faba* and the *E. europaeus* were moved to a net cage situated in the greenhouse on the 7<sup>th</sup> of May for rearing in a more controlled environment, where they were provided additional *V. faba* plants to colonize freely in a protected surrounding.

One week before the experiment, ten bean plants were chosen which were of the approximately same size, these plants will be more carefully described below. One AF was reared in each plant. The aphid was placed on one of the top leaves and left to wander to a place on the plant of its choosing. Each *V. faba* was controlled to be pest and predator free before the rearing of AF. Then, the plant was bagged using a plastic bread-bag, allowing gas and vapour exchange through pores and sealing off insects from entering or escaping. The bagged bean plant with the aphid on it was then marked out and put back in the same greenhouse, at the same place it had been growing before rearing the aphid on it. This process was done during three consecutive days to obtain thirty plants with thirty AF colonies that had been reared for exactly seven days prior to their use in the experiment.

#### *Dysaphis plantaginea*

The rosy apple aphid or RAA was collected from potted apple trees, *Malus domestica* var. Aroma, the same group of trees that were used in the experiments and where the ant-nests came from. Therefore, the RAA is also assumed to originate from the vicinity of the different orchards where the trees were previously situated, since the RAA oviposits on the fruit spurs and young shoots in the autumn and eggs hatch in the spring.





**Illustration 3:** *Dysaphis plantaginea*. Photo by Zapote

- Day one, RAA originated from Helenelust-, Juleboda-, Mandelmann- and Orelund-orchards.
- Day two, all RAA originated from Dammstorp-orchard
- Day three, RAA originated from Helenelust-, Orelund-, Källagården- and Dammstorp-orchards.

Aphids were collected for the experiment by removing the leaf, spur or shoot where they resided, and put into a net-bag that is insect-proof, or into a two ml plastic Eppendorf-tube for shorter periods. Thereafter, single RAA adults were chosen and put into clip-cages (described on page 10) by using the small blunt-side of a pocket-knife's screwdriver. The clip-cage were then placed on an apple tree that was going to be used in the experiment, precisely one week in advance. Each aphid was placed on a lower branch on a leaf that was slightly shaded, and close to the stem. Remaining RAAs that were collected from the trees, were mass-reared on two nurturing trees located in the greenhouse to provide back-up aphids. There they were put into insect rearing sleeves (MegaView Science Co., Taichung, Taiwan) free to roam and choose their location. The RAA and trees that came with them were also situated in the same greenhouse as previous insect-material.

## 3. 2 Plant material

### 3. 2. 1 *Vicia faba*

*Vicia faba* cv. Gloria, or broad bean was chosen as a host-plant for the *A. fabae*. Pots, ten centimetres in diameter, were filled on the 15<sup>th</sup> of April with Hasselfors garden<sup>®</sup> KRAV-certified vegetable substrate (grönsaksjord, Örebro, Sweden). 120 bean plants were planted on the 17<sup>th</sup> of April, using seeds from Impecta<sup>®</sup> (Julita, Sweden), giving them approximately six weeks to grow prior to experiments. Thirty of these plants were chosen out of similarity in size and vigour, and used for the experiment (**Illustration 4**).

The beans were cultivated in a greenhouse with a climate controller not allowing the temperature to go below twelve degrees Celsius and not above thirty. The beans were not given any added nutrients during the cultivation, only water when needed.



**Illustration 4:** *Vicia faba* plants growing in greenhouse prior to experiment. Photo by author.

### 3. 2. 2 *Malus domestica* cv. 'Aroma'

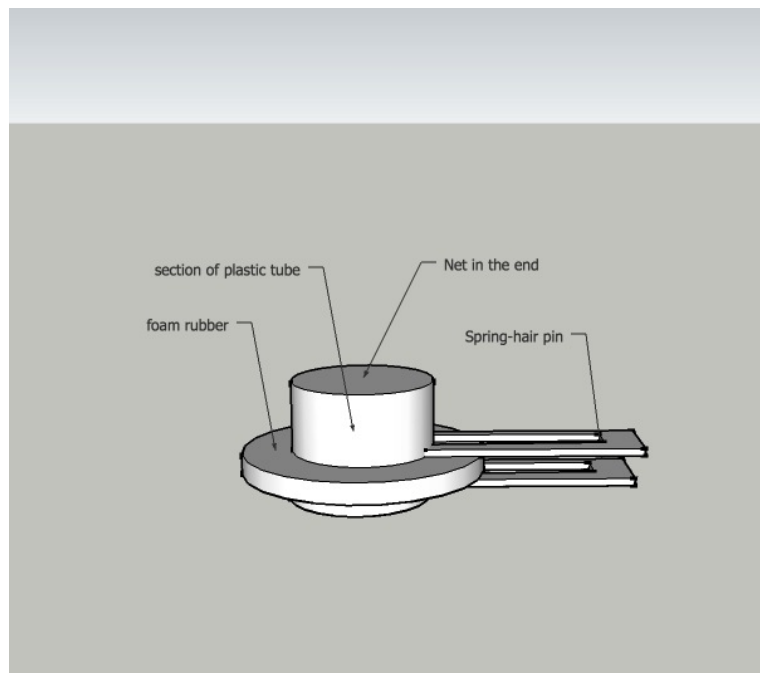
Trees used in the experiment originate from Stångby nursery (Lund, Sweden). The variety of apple used was 'Aroma', grafted on A2 rootstock. All trees were potted in 450 mm diameter plastic pots and all *M. domestica* were three years old. Trees were pruned after instructions by Marco Tasin (SLU, Alnarp), six weeks before the experiment was carried out.

One week before the experiment, ten trees were chosen and removed of visible insect pests and predators by hand to make sure it would not bias the result of the experiment. All trees were then placed inside the same greenhouse as above, in the same conditions as beans and insect material. This was done for three consecutive days, resulting in a total of thirty trees from the 25<sup>th</sup> to 27<sup>th</sup> of May. All potted trees were treated with Loxiran® 'Myr Effekt Pulver' (Neudorff, Emmerthal, Germany) an ant killer with active ingredients consisting of natural pyrethrins (*Chrysanthemum cinerariaefolium* extract) that was distributed around or on ant nests on the same day that the trees were brought into the greenhouse. This was done to make sure no ant nests existed in the potted trees when the experiment was run. No nutrients were added, only water when needed.

### 3. 3 The set-up

#### 3. 3. 1 Set-up material (hardware)

- Starving boxes: Cardboard boxes (576 × 346 × 407 mm) that were taped shut using packing-tape, both from the inside and the outside to make sure no ants could escape or forage for food outside of the box during the starvation period.
- 125 litre black plastic bags
- Clip-cages: Made from two small cross-section pieces of plastic pipe, sealed off in one end with nylon netting and rimmed on the other end with foam rubber. The clip-cage shuts by being adhered to a spring loaded hair-clip, retaining insects inside and hindering external predators to enter (**Illustration 5**).



**Illustration 5:** A clipage, used for rearing aphids on leaves.  
Illustration made by the author.

- 85 litre masonry buckets
- 90 mm wide wooden bridges

### 3. 3. 2 Description of the set up

The potted tree with the established colony of RAA was put into a masonry bucket that thereafter was filled with water. The *V. faba* was removed from its protective bag and planted into the pot where the apple-tree resided by removing soil and roots in the pot and replacing it with the living bean-plant. Beans that needed stability were trellised with a wooden stick. The tree and the bean were made sure not to touch one another. Clip-cages were removed from the leaf where the RAA was established.

Ant nests were lifted out of the starving-boxes every morning at 08:00 o'clock. The nest was removed from its pot, thereafter the root-lump containing the nest was put into a black plastic bag and thereafter back into its pot. This was done to make sure that the ant nests were not flooded during the experiment. The pot with the nest was then put in a masonry bucket like the tree. Also this bucket was filled with water, in both cases to confine ants to the set up, and shut out external insects. The two pots were connected with a wooden bridge at the start of the experiment, at 10:00 o'clock every day. The bridge was situated so that the distance to the tree and the bean-plant would be equal, not to bias the experiment. All trees were set up so that they did not touch each other, and were moved minimally to not disturb or stress aphids.



**Illustration 6:** Image of the set up, potted apple-tree in masonry-bucket. Pot with ant-nest and connective bridge. Illustration by the author.

### 3. 4. How the experiment was carried out (methodology)

Since the experiment was carried out in a greenhouse, it was considered a semi-field, semi-lab environment. Insect material was left to develop in a fairly un-controlled setting, light, temperature and humidity was directly related to outside conditions. The aphid populations were not standardised by numbers, but rather in time. All aphids were reared and left to multiply exactly one week in advance of the experiment. This resulted in varying colony sizes. The colonies however were paired to match in sizes as much as possible, and should therefore not affect the outcome.

The quantitative measurement that was taken in to account was number of ants on each colony of aphid, attending either the RAA or the AF. This was the basis for estimating qualitative preference of the ants.

#### 3. 4. 1 Number of replicates

The amount of repetitions chosen to carry out this experiment was a minimum of  $10 \times 3$  replicates for a statistically solid ground.

### 3. 4. 2 Timeframes

It was decided to run ten replicates a day for three consecutive days. The experiment started at 10:00 each day, running for the entire day, until 20:00, with a count of ants on aphids every two hours, at 12:00, 14:00, 16:00, 18:00 and 20:00. Counts were taken on the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>d</sup> of June, spring/summer of 2015. In the first thirty trees and beans prepared for the original days of experiment, twenty-three sets of plants were usable since establishment of aphids failed in seven cases. Another seven replicates were carried out on the 11<sup>th</sup> of June to make up the thirty replicates. The additional seven replicates were carried out in the same greenhouse with the same setup, and fall under the same description as above.

### 3. 4. 3 Weather and temperature conditions

Temperatures during the days of the experiment were ranging from 13.3°C to 28°C in the greenhouse. Weather was mostly sunny without clouds except for a few hours on the 2<sup>nd</sup> of June that had rain.

## 3. 5 Statistical analysis

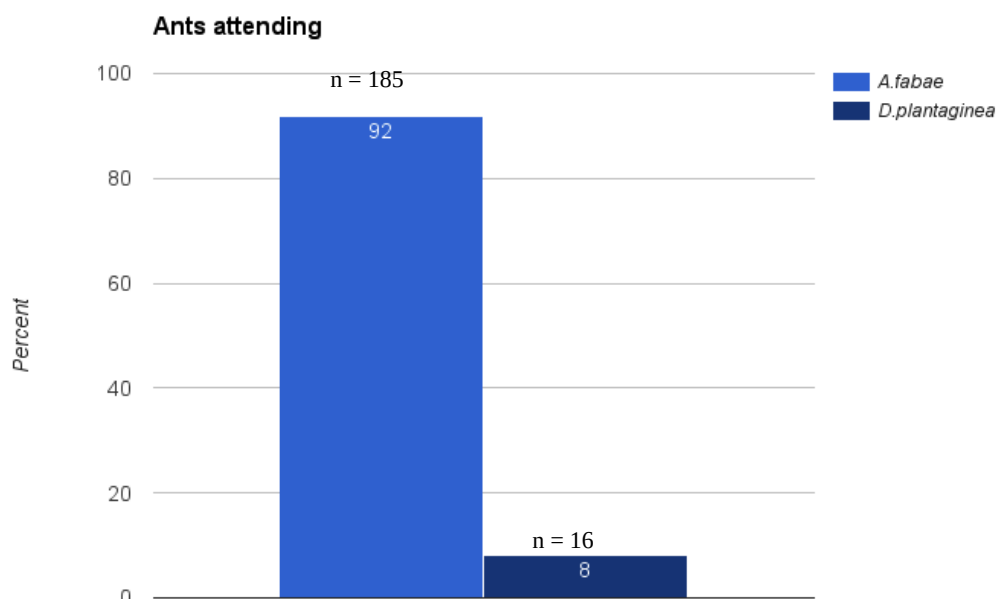
Minitab 17. 2. 1 as well as Minitab Express 1. 3 were used for the statistical analysis. To see weather difference in ant attention intensity was achieved the attention of both aphid species were compared with each other, in each time-point and over days. First each group of samples was checked for normality of distribution using a Kolmogorov-Smirnov test, and all groups of samples were established as normal. This resulted in use of a Student's *t*-test for groups of each point in time checked, for example the sample of ants attending AF at 14:00 were tested against ants attending RAA at 14:00 to see whether there was a difference between the aphid attractiveness in terms of amounts of ants attending them.

The time-dependent analysis was conducted to see how the attention changed over time, and what effect time had on the experiment. First all sample were 'Log10 (x+0.5)' transformed as suggested by Yamamura 1999 due to the many counts of zero in samples (Log10 of zero returns an error-message since the Log10 of zero is infinity). Thereafter a regression analysis was carried out with the count of ants at different times as the response factor and time as a predictor of the response, for each of the aphid species respectively.

Diagrams were made using Minitab 17. 2. 1 and Google-sheets.

## 4 Results

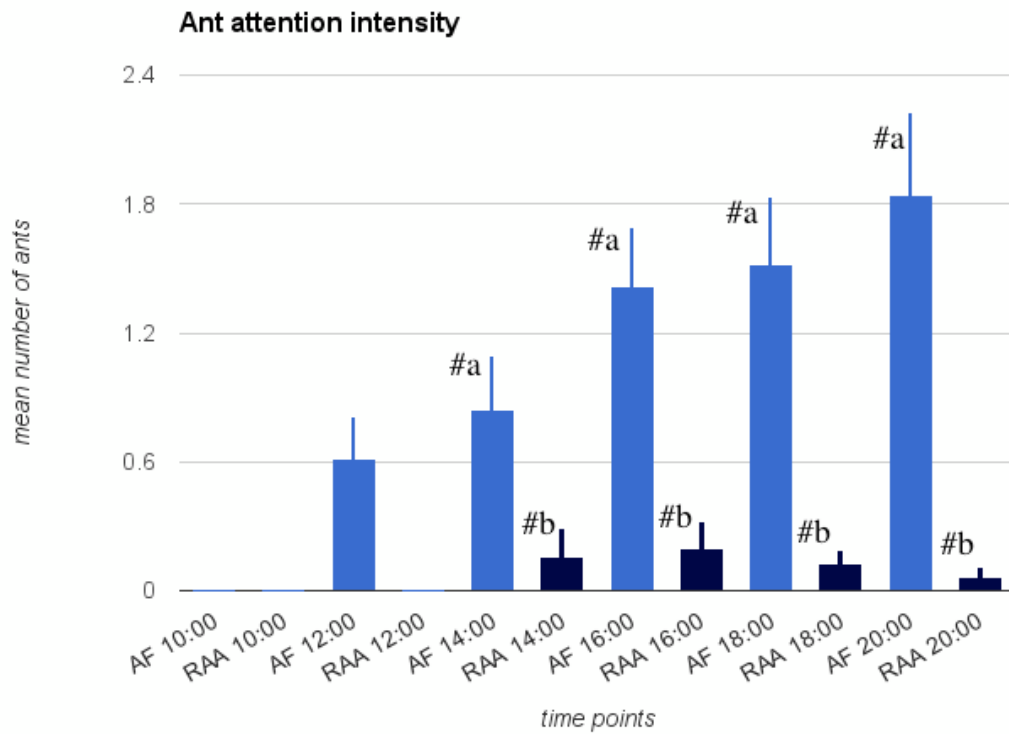
From the total of thirty replicates, an amount of 201 ants attended aphids in the experiment. 185 were attending AF and sixteen RAA, respectively. As seen in **figure 1**, that is a 92 - 8 % ratio between the two species.



**Figure 1** Percent-rate of which aphid ants attended during the experiment. *n*, amount of ants attending.

### 4. 1 Ant attention intensity

A mean number were derived from each sampling time for all the replicates. This is used to give a mean number of ant attention on that specific time of sampling. From this it is derived at what intensity ants attended the two aphid species (**figure 2**). Groups in each time-point that have a different letter, differ significantly. In the case of the 12 o'clock sample there could not be a significance established, since there was nothing to compare AF attention with (no ants attended RAA at that time).



**Figure 2** Mean number + SE of *Lasius niger* attending aphids at given time. Samples not sharing a letter are significantly different at  $p < 0.05$  (Kolmogorov-Smirnov followed by Student's t-test).

## 4. 2 Time effect

**Table 1:** Regression analysis values of the attention of the two aphids.  $n$ , number of samples. Significance at  $p < 0.05$  (simple regression-test)

| Aphid  | $r^2$ | P-value of time-variable |
|--|-------|--------------------------|
| <i>A. fabae</i> vs. time ( $n = 155$ )       | 6.86% | 0.0010                   |
| <i>D. plantaginea</i> vs. time ( $n = 155$ ) | 0.58% | 0.3482                   |

The regression analysis shows the chances of multiple consecutive samples being the same over a time-period. If P-values are below 0.05 it is very probable that the results are changing over time, and this is the case of *A. fabae*-attention (**Table1**). The p-value of *D. plantaginea* on the other hand does not show a significant difference over time, and it can not safely be stated that the attention of the rosy apple aphid will change over time.

The  $r^2$  describes how well the data fit a regression model, how big chances are that a sample is situated on the fitted line in a scatter-plot of all the samples. As seen in **table 1** neither of the two have a very high percentage, and this will be discussed further below.



## 5 Discussion

### 5. 1 Discussion of statistical results

#### 5. 1. 1 Ant attention intensity

**Figure 2** is the soundest evidence of this experiment's outcome. It speaks loudly for itself, both visually and statistically. Needless to say, the result was in favour of AF, performing exceedingly better than RAA. Interesting was that the attention did not decline in the evening in the case of AF but rather increased constantly, it was expected that the ants would not be active during the night due to previous studies by (Fischer, Hoffmann, and Völkl 2001, Engel et al. 2001 and Fischer et al. 2002) who all based their studies on *L. niger* being diurnal. My theory is that the experiment was quitted too early in the evening to see a decline of ants due to time of day.

#### 5. 1. 2 Time effect

$r^2$  problems, or a low goodness-of-fit in the regression analysis in this experiment seem to be that the mass of samples containing zero-counts outnumber the above zero counts, by far in the case of the RAA. The many counts of zero has an inflation-effect that makes this model somewhat un-suited to analyse the data obtained,  $r^2$  tells us what the chances are of a sample to fall onto a fitted line in the model, the probability of samples following a pattern. Obviously this is not the best way to model this data and more advanced analysis would have to be performed to increase the fit of the model. However the  $r^2$  is much higher in the case of AF telling us that the fit is better there than in the case of the RAA. More so it gives an idea of how the regression analysis pan out when looking at the P-value that describe, in this case, the probability of change from one sample to another. Indicating if a line is going upwards (or downwards), or in the case of the RAA, not significantly changing at all, a flat line. However as seen in **figure 2** the attention of ants seem to be declining in the rosy apple aphid. In this study we content ourselves in the fact that there is significance in change during time in the AF, and not in the RAA.

## 5. 2 Discussion of methodology

The reason for choosing this semi-field set up for the experiment was to make an as realistic study of the mutualistic behaviour of ants and aphids as possible. It was hypothesized that the real strata of an orchard, with the height of the tree would not only be more realistic than an equal-distance test or a no-choice test, but could give a higher effect of the experiment, since it is stated in Devigne and Detrain (2005) that ants prefer food sources close to the nest. However, Detrain et al. (2010) changed that thesis, saying that ant scouts come over carbohydrate sources by chance and then decide whether or not to exploit the source. Stadler and Dixon (1998), Fischer, Hoffmann, and Völkl (2001) and Detrain et al. (2010) show that ants do not only choose the closest aphid, but on basis of quality and quantity of honeydew. This is in line with what was observed in this study, unrecorded but noted that ants quickly search the entire set up area including the tree. It seems safe to say that the honeydew quality and/or quantity are the major attractants for the ants in this experiment, and not the spatial distance to the food-source. When setting up the experiment, the spatial distance was considered a factor to take into account, this resulted in a decision that the rosy apple aphid should be placed in close vicinity to the trunk of the apple tree. The application of the RAA by clip-cage impaired the aphids choice of location for formation of a colony, but were rather forced to stay where they were put, this leads to a possibility of unfavourable placing of them, not providing the optimal conditions for growth. In comparison, the entire *V. faba* plant was enclosed in a ventilating bag, allowing the AF to choose their dwelling on the plant, which usually was at the very tip of the bean-plant. On the other hand, colony-sizes were paired to be as equal in size as possible (**table 2**). There is a representation of colonies of both aphid species that are larger than the other in the same sample, yet the results still show an advantage for AF (**figure 2**). This would also suggest that the colony size did not have a great effect on the outcome in this case.

**Table 2:** Proportion of colonies. *Dysaphis plantaginea* vs. *Aphis fabae* (*Dysaphis plantaginea* in red numbers and *Aphis fabae* in black).

| Day | Tree 1 | Tree 2 | Tree 3 | Tree 4 | Tree 5 | Tree 6 | Tree 7 | Tree 8 | Tree 9 | Tree 10 |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|
| 1   | -      | -      | -      | 14/24  | 5/12   | 15/18  | -      | 18/21  | 12/12  | 33/28   |
| 2   | 32/22  | 8/13   | 8/19   | 22/22  | 16/16  | -      | 15/18  | -      | 14/12  | 7/10    |
| 3   | 11/15  | 23/25  | 40/19  | 10/11  | 19/18  | 14/13  | 9/24   | 21/13  | -      | 17/14   |
| 4   | 30/27  | 33/30  | 24/17  | 17/11  | 14/9   | 39/30  | 40/24  | 19/11  | -      | -       |

Confining the RAA within the clip-cage during their week-long development seemed in many cases to cause them to scatter when released from the clip-cage at the beginning of the experiment. Resulting in aphids on the move, and therefore not producing honeydew. Honeydew quantity is a factor that is described by many to effect the ant choice of mutualistic partner (Bonser et al. 1998, Stadler and Dixon 1998, Detrain, Deneubourg, and Pasteels 1999,

Völkl et al. 1999, Fischer, Hoffmann, and Völkl 2001) Colony size is a given factor to total amount of honeydew produced, and therefore smaller, or scattered colonies should produce smaller pooled quantities. The scattering was however not the case in all colonies, and their initial movement resulted in a settling on other nearby shoots.

Noted should also be that on tree nine on day two ants attended no aphids, but instead the flowers of the tree. Approximately ten flower clusters were visited by twenty-five ants collecting nectar as a source for carbohydrates (**Illustration 7**). This was only seen in this sample.



**Illustration 7:** Ant feeding from an apple-blossom. Photo taken by the author.

## 5. 3 Discussion of aphid properties

The foremost sugar that ants are attracted to is melezitose (Völkl et al. 1999, Fischer, Völkl, and Hoffmann 2005, Detrain et al. 2010) an aphid specific sugar. It is an area of some uncertainty of what aphids can be ascribed to this signature sugar, Völkl et al. (1999) and Detrain et al. (2010) have concluded that *A. fabae* produces small, or no amounts of melezitose whatsoever. Vantaux, Billen, and Wenseleers (2011) and Vantaux et al. (2012), show that there is more to the black bean aphid than first thought. By both genetic analysis and honeydew analysis it is estimated that there is a larger diversity in the expression of aphid behaviour, and that up to half of all *A. fabae* do not produce melezitose, even if attended by ants. It can be concluded that knowing the properties of different subspecies of *A. fabae* is difficult, even if we do know the sub-species, we cannot be certain if it produces the

melezitose, key to attracting ants. But Vantaux et al. (2012) also proves that the ant-attention is not lower in the non-melezitose producing clones of *A. fabae*. This can only fuel the fact that we know too little of the mutualism between ant and aphids, and key factors influencing this relationship. Fischer, Völkl, and Hoffmann (2005) on the other hand have proven that subspecies of *A. fabae* produce melezitose, ranging from 20% in the lowest case, to 80% in the highest melezitose-producing subspecies. This was recorded from aphids feeding from *Cirsium arvense*, the creeping thistle, which for obvious reasons as a difficult pest-plant would not suit this endeavour.

Although the melezitose-production of *D. plantaginea* is yet to be researched, it is known to be an ant-attended aphid (Nagy, Marko, and Cross 2007, Miñarro, Fernández-Mata, and Medina 2010, Nagy, Cross, and Marko 2013). Miñarro, Fernández-Mata and Medina (2010) showed in their study that *D. plantaginea* in fact were less attended by ant mutualists in comparison to an *Aphis* sp. aphids, supporting the findings of this experiment. The seeming abandonment of *D. plantaginea* is in fact in line with current ideas of ant mutualists abandoning a lesser productive aphid (Fischer, Hoffmann, and Völkl 2001).

Admitting that no honeydew-analysis was conducted for this experiment in either aphid, the results would point towards *A. fabae* being the more attractive mutualist for ants. If this result could be quantified by quality or quantity of honeydew, or both will be left for future investigation.

## 6 Conclusion

It was discovered in this study that the black bean aphid is a better suited ant-mutualist than the rosy apple aphid. With a total amount of 92% of all ants attending the black bean aphid, and the seeming action of ants abandoning the rosy apple aphid out of preference of the black bean aphid, it seems most likely that the rosy apple aphid would be left un-guarded against their natural enemies. As a result of this it has been proven by Nagy, Marko, and Cross (2007), Stewart-Jones et al. (2008) and Nagy, Cross, and Marko (2013) that the mortality of the rosy apple aphid is significantly higher in colonies that are left un-attended by ants. This is also the case in other studies focusing on other aphids, and the consensus is that, aphids that are not protected by ants suffer significantly greater loss.

It can be concluded that this application of a functional element, the aphid-infested bean plant, would in combination with a healthy predator population, significantly elevate rosy apple aphid predation by means of re-directing ant attention, and therefore reducing ant protection of *D. plantaginea*. Although an application in real orchards is yet to prove this point.

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