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Final report

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An Innovative IPM Approach for an Effective and Low-Risk Control of Aphids of Fruit Crops

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Part 1.1: Summary/Abstract

In an ecosystem approach, we aimed to increase biocontrol of aphids by sustainable management of the ecosystem interactors, including ants, aphids and natural enemies. Ants protect aphids, because of the honeydew reward. A carefully composed alternative reward (Ant-Stop) strongly diverted ants from tending rosy apple aphids (RAA). Accordingly, the number of colonies and aphids in colonies was significantly reduced, and colonies collapsed early. Concurrently, natural enemy presence increased in RAA colonies increased. Volatiles identified from RAA colonies and tested in the field, synergized this and increased the presence of natural enemies in aphid colonies. In cherry, suppression of the black cherry aphid was clear, yet not sufficiently strong. The research made significant progress toward a novel, entirely sustainable method to control RAA, the most important apple pest in Europe. Translation of this innovation into other aphid-ridden production systems needs further research.

Svenska: I en ekosystemansats försökte vi att öka biokontroll av bladlöss genom en hållbar förvaltning av ekosystemets olika aktörer. Myror skyddar bladlöss mot naturliga fiender i utbyte mot honungsdagg. I en fältförsök i äpple, en noggrant sammansatt alternativ socker ’belöning’ (Ant-Stop) avledade starkt myror från att sköta rosenbladluskolonier, och därmed öppnade nischen för naturliga fiender. Detta åtföljdes av en kraftigt minskad tillväxttakt och en tidig kollaps av rosenbladluskolonier. Lockbeter av dofter identifierats från bladlösskolonier gav en synergieffekt (Predator-Pull), och ökade den biologiska kontrollen. I körsbär fanns det en klar effekt på den svarta körsbärsbladlusen, men inte tillräckligt stark. Forskningen har tagit ett viktigt steg mot en ny och helt hållbar metod för att bekämpa rosenbladlusen, den viktigaste skadegöraren på äpplen i Europa, och motiverar vidare forskning om hur innovationerna kan översättas till andra odlingssystem som lider av bladlusskador.

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Part 1.2: Main report (max. 10 pages)

Introduction

Due to EU regulation broad spectrum insecticides such as neonicotinoids are being rolled back in horticultural production systems (EFSA, 2018), given their strong externalities in the ecosystem. Well known effects include high toxicity to pollinators and natural enemies, as well as a breakdown into potentially hazardous metabolites (Christen et al., 2016). A time-limitation of their application is already in effect in the national action plan of a number of EU countries such as Italy, France and Holland.
While this is certainly promoting and accelerating the development of sustainable horticultural production, alternative control measures are few. In Sweden, aphids of horticultural crops such as apple, cherry, pear, plum and vegetables are often controlled by the above-mentioned insecticides. Given the disincentive to develop new aphicides due to the increasingly strict regulation, as well as the rollback of existing ones, sustainable alternatives need to be developed to provide efficient and low-impact aphid control options. This is in line with the current guidelines for IPM in Europe (Barzman et al., 2015).
Traditionally, often pests have been studied isolated from its context, its ecology. This not only leads to 'solutions' that may be at odds with ecosystem health, but also means losing out on opportunities provided through the ecosystem itself. A close look at ecosystems surrounding aphids show that interactions are dominated by ants. Indeed, many ants species are known for myrmecophily: aphids maintain a close association with ants, which provides protection in return for honeydew, an abundant source of nutrition (Stadler and Dixon 1998, 2005). As myrmecophily poses a problem in our agroecosystems by reducing aphid vulnerability to natural enemies, severing this relationship may reduce protection and increase exposure to biocontrol. Indeed, exclusion of the black garden ant, *Lasius niger*, in apple trees by using sticky barriers on the bark of trees (Stewart-Jones et al. 2008; Minarro et al. 2010; Nagy et al. 2013), or diverting *L. niger* using aphid-infested plants or sugar baits may reduce aphid colonies and/or increasing natural enemy presence (Nagy et al. 2015; Pålsson et al. 2020). However, these studies showed effects on small-scale, e.g. the level of individual colonies, and don't provide a full scale, spatio-temporal picture of the effects on population dynamics of aphids, ants and the guild of natural enemies. Further, their impacts and limitations in biocontrol of aphids in ecologically intensified orchard settings has not been tested. This is increasingly important in the light of conflicting literature about ecological intensification and biocontrol of aphids (Albrecht et al. 2020).
In addition to the possibility of diverting ants to increase access of aphid colonies for natural enemies, attractants could possibly also increase the presence and ecosystem services provided by natural enemies. Natural enemies are well known to orient to diverse signals, particularly those induced by herbivory. These so-called herbivore induced plant volatiles (HIPVs) are released by the plant and constitute a 'true' signal which natural enemies (parasitoids and predators) exploit to locate their hosts. Most agricultural and horticultural crops release HIPVs, and compound range from green-leaf volatiles released on the site of attack (GLV's) to terpenoids (often systemically released by the whole plant), as well as more specific signaling compounds, some of which can even 'warn' neighboring plants of attacks, such that they are also raise their defense responses. Enhancement of biological control through attraction of beneficials via HIPVs has been well-described in literature since long (Turlings et al., 1993), and primarily used in the context of plant breeding, for instance through highlighting the importance of varieties that show a sufficiently high defense response to herbivore attack. However, translation of the concept into application has been delayed as regulatory incentives for changing farmer practices were limited (Penaflor and Bento, 2013). Thus far, no synthetic HIPVs released by aphids of fruit trees were tested as synthetics in literature as predator or parasitoids attractants for aphid biocontrol. If and how HIPVs can be used to increase ecosystem services rendered by natural enemies, through 'luring' them into the apple tree has been very little tested.

Objectives: Here we studied the chemical ecology of multitrophic interactions surrounding aphid colonies, with as aim to develop a new sustainable strategy for reducing the impact of aphids in apple (rosy apple aphid, Dysaphis plantagineae, primarily), and the black cherry aphid (*Myzus cerasi*). By developing and combining two intervention methods, one of which targets ants, the other natural

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enemies, the strategy aims to synergistically increase biocontrol of aphids without the use of pesticides. In addition, the potential impact of ecological intensification on enhancing biocontrol of aphids is evaluated.

Translated into objectives: 1). Identification of the optimal blend of sugars, amino acids and protein to divert ants from tending and protecting aphid colonies, 2). Identification of HIPVs released by apple trees infested with the rosy apple aphid *Dysaphis plantaginea.* 3). Identification of blends that attract natural enemies to enhance ecosystem services by attracting NE to RAA colonies, 4). Assess the field efficacy of optimized ant diversion solutions, and natural enemy attractants, in reducing the number of aphid-tending ants, population dynamics of various aphid species (the rosy apple aphid, the green apple aphid and the black cherry aphid), and augmenting the number of resident natural enemy species (larvae of the ladybird beetles, green lacewings and syrphid flies). 5). The effect of attractants on occurrence of resident natural enemy species, and the population dynamics of the rosy apple aphid.

Materials and methods

Laboratory and semifield experiments

Unraveling the composition of honeydew and optimization of Ant-Stop baits: Honeydew of *D. plantaginea* and *A. fabae* was collected, the composition of sugars and amino acids was established Ion Chromatography - Pulsed Amperometric Detection – Charged Aerosol Detection (IC-PAD-CAD) and high precision liquid chromatography with fluorescence and diode-array detectors (HPLC-FLD-DAD), respectively. The attractiveness of a recomposed honeydew mimic attractiveness was tested against different combinations of sucrose (4 and 20%, Nordic sugar A/S, Copenhagen, Denmark), melezitose (4%, Merck KA, Darmstadt, Germany), amino acids (in ratios found in the honeydew of *D. plantaginea,* Pålsson et al. 2020) and protein (egg protein, and corn protein) following demonstrations of increased attractiveness of protein-laced baits (Madsen et al. 2017). Tests were done in the laboratory with lab colonies of *L. niger*. In the field, in an apple orchard (Alnarp, Sweden) five to seven different colonies of ants were selected on the basis of activity levels. The number of visiting ants on each treatment were counted multiple times throughout the day. The most optimal composition was used for large scale field trials in suppressing aphids through diverting ants.

Evaluation of formulations: Baits were tested in both liquid formulations (vials with cotton stoppers) as well as SPLAT (ISCA Technologies, CA), the latter offering resistance against washing out and a potential for pray applications. Odors were added to baits to assess if the attractiveness of baits could be augmented through associative learning by ants. Data of the experiments with SPLAT and odors are not elaborated on in this report, as neither of them significantly increased the preference or ant visitation of the baits.

Analysis of honeydew and attractants for natural enemies: Plant VOC were sampled using adsorbant collections from single branches in the field and subsequent characterization using CLSA-GC–MS, as well as ‘live’ sampling of diel patterns of volatile release using PTR-ToF-MS using potted plants (Badra et al 2021). Samples from branches containing RAA colonies were compared to branches from uninfested control trees. Based on the volatile profiles, lures were composed and the behavioral response of green lacewings tested in the wind tunnel. Lures were then composed in SPLAT and prepared for field tests, with literature data supporting fine-tuning of the blend. Literature data (e.g. Heuskins et al. 2012, Verheggen et al. Stökl et al. 2012..) formed the basis for a syrphid fly lure, which was embedded at 1% at a 1:1 ratio of six mono and sesquiterpenes, as well as *E*-3-hexenol.

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Field experiments

Locations: Experiments in apple were carried out in Alnarp (SLU, organic orchard) and Solnäs Gård (Kivik Musteri, Bjärred). Ant diversion experiments with collaborators in France, Belgium and Poland, were planned, but did not materialized, due to several circumstances. Experiments in cherry were carried out in an IPM orchard in Costasavina (Pergine, Italy) at 506 m above sea level. Farmers agreed to postpone sprays in the experimental sections against aphids until after completion of the research.

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***Baits used**:* For the field tests, we did not include protein in the bait as it affected field life (decay of protein), and could reduce the ecosystem services rendered by ants as predators of pests (as well as rosy apple aphids later in the season, through a reversal from commensal to predator). The lack of protein could be compensated for by increased sucrose concentrations, and therefore preferred. Although amino acids only slightly increased the attractiveness of the most attractive Ant-Stop formulation (Fig 1), they are important tastants for insect. To avoid potential habituation to sucrose alone, a mix of amino acids found in honeydew was therefore. In addition, a series of experiments was done where the diversion was composed of bean aphid colonies (*Aphis fabae*) on bean plants (*Vicea fabae*), in an apple-bean intercropping setting both in the greenhouse and the field.
Lures used: Odor lures for lacewings and for syrphid flies were embedded in SPLAT. Each tree was equipped with a single dollop, which was placed on a waterproof plastified carton strip, looped around a branch with the ends stapled together. Lures were replaced every 10 days.
Ant mapping: To assess spatial diversity in ant activity prior to intervention, activity of ants throughout the experimental orchard in Alnarp was mapped across weeks using a 20% sucrose solution. Ant activity was superimposed on an orchard map and used in further evaluation of the results of ant diversion experiments.
Aphid, ant and natural enemy monitoring: The establishment and growth of *D. plantaginea* and *A. pomi* colonies were carefully monitored from May - July. Artificial honeydew following detection of aphid colonies. Aphid colonies were marked with a unique identifier, and monitored, counting the number of aphids, ants, and larvae of three natural enemies, Coccinellidae, Syrphidae and *Chrysoperla spp*., as a proxy for pressure by the natural enemy guild. Monitoring of *A. cerasi* and associated ants, was done weekly in selected trees, in Italy. Monitoring in apple continued until most RAA colonies in control trees collapsed (July). In cherry the experiment was terminated after six-week intervention.

Data analysis

Data on ant preference for combination of amino acids and sugar were pooled across observations for each replicate and for each of the pilots (Pilot 1-3). Data was fitted using a generalized linear model with a poisson distribution, or a negative binomial distribution. Density maps of monitored ants, ant attendance, natural enemies and aphid density, were created after normalization for unequal distribution of trees (dead and pollinator trees in the orchard). Normalization was done through interpolation to an equidistant matrix. The interpolated data was then superimposed on a satellite image downloaded from Google's api using "ggmap". For plotting the number of aphids, number of aphid colonies and number of natural enemies over time, days since the start of experiment (date) was used as the explanatory variable in a cubic polynomial model, fitted with a poisson distribution. The number of ants and natural enemies in each aphid colony was summed across dates and modeled using a generalized linear mixed model, fitted with a negative binomial model with each tree as random effect. For modeling, the natural log of aphids versus tending ants and natural enemies, a cubic polynomial model fitted with a poisson family was used with the natural log of aphids as the explanatory variable. For the primary component analysis (PCA), the package "tidymodels" was used and the data was centered and scaled. The variance contributed by each primary component was calculated from the standard deviation and divided by the total variance. Envelopes for groups were calculated using the Khachiyan algorithm. All visualisations, data manipulation and organisation of the data was done using "tidyverse" (Wickham et al. 2019).

Results

Composition of honeydew, preference of ants, and formulation of Ant Stop.
During the course of this project a series of questions were tackled sequentially to develop a sustainable alternative to control the aphids in fruit production. As a critical component to diverting the commensals of aphids, ants, we investigated which sugars and amino acids aphid honeydew was composed of (Fig. S1), and which were most preferred by the aphid commensal, *L. niger* (Fig. 2) were most preferred. Whereas fructose and sucrose were main sugar components of honeydew, *L. niger* preferred strongly combinations with the trisaccharide melezitose. To overcome the need of using melezitose, which is very expensive, we increased the sucrose concentration from 4% (as in honeydew) to 20% (Fig. 1B),

with added amino acids increasing the attractiveness slightly (Fig. 1C). Formulation in SPLAT as slow release matrix, significantly reduced the attractiveness of sugar and amino acid combinations (Fig. S2). Egg protein, previously reported as ant attractants, neither increased attractiveness of the sucrose solutions. Further experiment therefore only used sucrose solutions placed in vials with a cotton stopper.

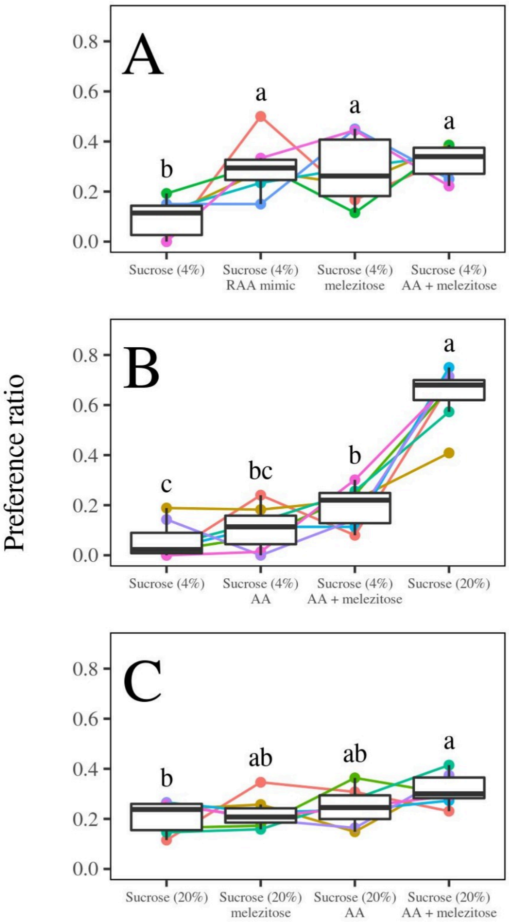

Fig. 1. Evaluation of preference of *L. niger* for different combinations and concentrations of sucrose, melezitose and amino acids. Each line connects the preference of a single ant colony for four compositions. Depending if the data was overdispersed, a glm model plotted with either a poisson (B) or negative bi-nomial (A,C). A) Sucrose 4% is tested alone or combined with either a *D. plantaginea* mimic (RAA), melezitose or melezitose + amino acids (AA). B) Sucrose 4% tested alone, and in combination with AA or AA + melezitose against 20% sucrose. C) Sucrose 20% tested alone, and in combination melezitose, AA or AA + melezitose.

Further experiments were conducted to evaluate whether companion plants infested with aphids would divert ants from tending the rosy apple aphid colonies in apple trees. Indeed, *A. fabae* infested bean plants could lower ant tending significantly (Fig. S3) However, phenological asynchrony between both plants and their infestations made this route not practical for further experimentation and future application. Experiments using okra plants (*Abelmoschus esculentus*), whose pearl bodies are highly attractive for ants, was similarly constrained and was discontinued. Experiments with Vicea species

Effects of Ant Stop on ants, aphids and natural enemies

In subsequent field experiments we tested whether a formulation of sucrose plus amino acids (Ant Stop) could divert ants from tending aphids, and how this affects aphid colony dynamics, and the numerical response of natural enemies (Fig. 2). The results demonstrate that application of Ant Stop effectively diverted ants away from tending both the rosy apple aphid (RAA) and green apple aphid (GAA). Aphid colonies showed significantly reduced growth and collapsed significantly faster. In contrast, the presence of natural enemies in aphid colonies, particularly those of RAA (Fig S5) significantly increased, with those of syrphid and *Chrysoperla* larvae showing the most significant numerical responses. Of further importance is that the effects on aphid colonies and natural enemies depended on early application of Ant Stop. Application at or after the exponential growth phase of RAA colonies had a negligible effect on RAA colony size and RAA numbers or the presence of natural enemies (data from 2019, Fig. S4 left panels, Fig. S5 top panels). Similar results were obtained in 2022 (data not shown). Data across years also demonstrate that population levels of natural enemies varied considerably between years and between organic and IPM orchards.

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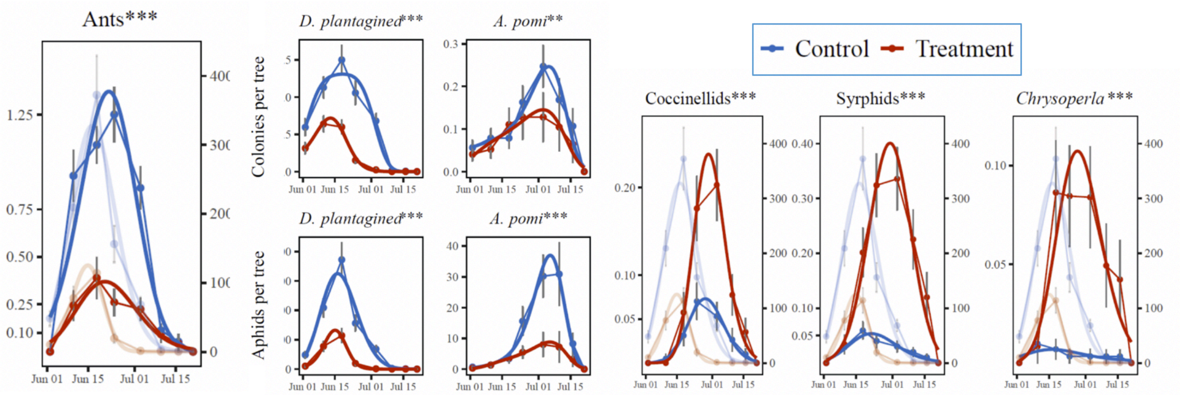

Fig. 2. Effects of placing Ant Stop at the base of each apple tree on the number of ants per aphid colony (left panel), the number colonies and total number of rosy apple aphid (*D. plantaginea*) and green apple aphid (*A. pomi*) colonies, and the number of resident natural enemies in aphid colonies (right three panels). Stars after taxa names denotes significance level (* < 0.05, ** < 0.01, *** < 0.001).

Identification and use of volatiles to augment the numerical response of natural enemies

To further augment the numerical response of natural enemies to aphid infestations in apple, experimental lures were designed based on literature data, as well as analysis of volatiles from herbivore-induced plant volatiles (HIPVs) from RAA colonies (Fig S6), and tested in wind tunnel and field tests. Lures for lacewings and syrphid flies formulated in SPLAT, as well as a floral lure that increases pollination, were placed one each in apple trees. This increased the presence of both syrphid fly and lacewing larvae in RAA colonies (Fig 3), although the effects differed between orchard type and apple variety.

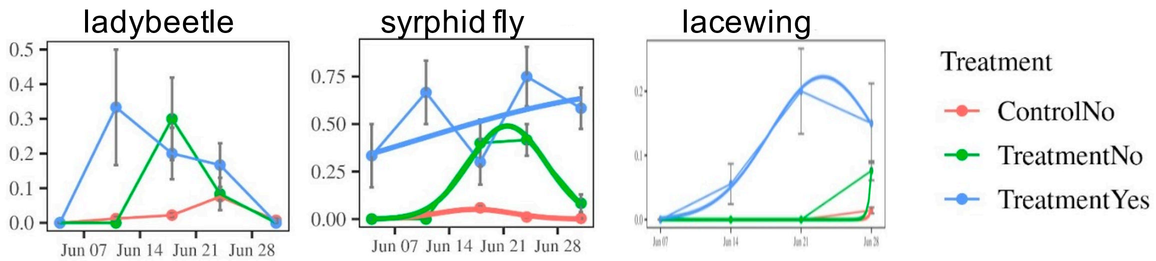

Fig. 3. Lures for natural enemies augmented the effect of ant diversion on the numerical response of resident natural enemies.

Effect of Ant Stop on black cherry aphid

We evaluated the potential of Ant Stop in other production systems through applying Ant Stop was applied in the early season in Italian cherry orchards. Ants tended BCA colonies significantly less in treatment trees, which reduced the growth of colonies (Fig. 4). However, the effects were much less pronounced as observed for *D. plantaginea*, and

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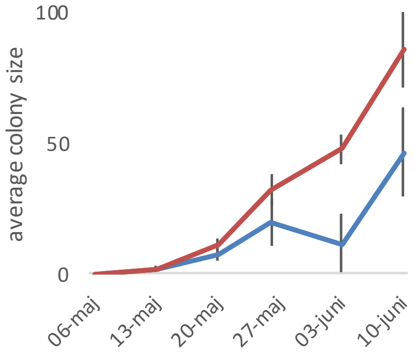

Fig. 4. Ant Stop reduces ant tending black cherry aphid (*A. cerasi*), and significantly reduced the average BCA colony size.

Discussion

The rollback of insecticides may endanger the economic sustainability of production systems. Research for ecologically sound alternatives delivers at an underwhelming rate. Whereas much research efforts provides useful insights into ecosystem functioning, how this can be translated into practice needs attention. This SLF grant enabled applied research on sustainable alternative control measures against aphids in fruit production. The results show that sustainable rebalancing some ecosystem interactions permit control of one of the most severe pests in apple, the rosy apple aphid, while simultaneously increasing populations of natural enemies. The intervention can conceivably be developed into a low-cost application that is fully compatible with organic production.
In recent years, much research has been channeled to ecological intensification, bringing nature back into our agroecosystems, as a means to restore ecological functions and biodiversity to harness ecosystem services in production (Tittonell 2014). In terms of pest management, ecological intensification aims to diversify agroecosystems to 'invite' natural enemies back and with them, nature's self-regulating, pest-suppressing abilities (Bommarco et al. 2013). However, increased biodiversity may not equate functional ecosystem responses to pests. In our perennial apple production system, myrmecophily obstructed biological control of aphids through hindering functional responses of natural enemies, in spite of ecological intensification. Only when *L. niger* was diverted, could the increased abundance of natural enemies be levered into successful biological control.
Indeed, on contrast to many studies in annual systems, in perennial cropping systems such as apple, blueberry, hops and strawberry, ecological intensification using flower strips significantly increased the abundance of natural enemies, but this had had negligible effects on aphid populations (Walton and Isaacs 2011; Markó et al. 2013; Calderwood et al. 2017; Campbell et al. 2017; Hodgkiss et al. 2019; Rodríguez-Gasol et al. 2019; Cahenzli et al. 2019; McKerchar et al. 2020). Clearly, abundance and functional responses of natural enemies of aphids are correlated under tilled annual cropping regimes, but not under perennial and no-till regimes, i.e. regimes with low soil disturbance that support ant colonies (Marti and Olson 2007; Baraibar et al. 2019). Besides important practical implications for ecological intensification for aphid control, it also highlights that ecological intensification should be accompanied by detailed studies on insect food web interactions and how these are, or are not, impacted by an increased biodiversity. Our study shows that in the perennial apple cropping systems, ecological intensification by itself did not support biological control of aphids. Number and impacts of natural enemies on both *D. plantaginea* and *A. pomi* were minimal, in spite of their abundance. Instead, myrmecophily upheld aphid colonies in an increasingly aphid-hostile ecosystem by obstructing functional responses of natural enemies, and completely blocking the enhanced biocontrol potential obtained through ecological intensification. Conversely, we show that diverting ants, which canceled out the functional response of ants to aphids, unlocked the functional potential of natural enemies.
Myrmecophily shapes predator guild dynamics. In response to disruption of myrmecophily, three important taxa of aphid predators, larvae of *Chrysoperla spp.*, Coccinellidae and Syrphidae (Völkl et al. 2007), showed model-type functional responses to aphid populations. It should be noted that these three

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indicator species served as a proxy for other species of natural enemies in the orchard, but whose presence was more transiently associated with aphid colonies and are harder to detect without intrusive and disruptive methods, e.g. Forficulidae (earwigs), predatory heteroptera species, parasitoids and spiders (Völkl et al. 2007). In earlier, small scale experimental studies in non-intensified ecosystems, trees were inoculated with aphid colonies and/or a few infested branches per tree were selected. Diverting ants using exclusion or alternative sugar sources increased pressure of natural enemies and resulted in reduced aphid colony number and size (Stewart-Jones et al. 2008; Nagy et al. 2013, 2015; Wäckers et al. 2017; Pålsson et al. 2020). We demonstrate that in spite of ecological intensification and high presence of natural enemies, functional responses to naturally established aphid populations require severing myrmecophilic relationships, such that the ecological stable state shifts in favor of natural enemies instead of ants.

Our study also aimed at increasing the functional response of *Chrysoperla spp.*, Coccinellidae and Syrphidae. We demonstrated that each of these resident larval predators showed a significant numerical response to Ant Stop. Clearly, the niche opening through disrupting myrmecophily shifted aphid food web dynamics and the relative importance of individual predators. Such changes following interventions need monitoring, and measures need adjusting to fit the phenology of pest and natural enemies to maximize ecosystem services, and may substantially differ between orchard type (organic versus IPM) and even between varieties (or position in the orchard), as our results demonstrate. Practical implications could for instance include the use of strips with early flowering perennials which particularly support Syrphidae to enhance enhancing aphid control in apple (Haenke et al. 2009; Hogg et al. 2011).

Furthermore, the effects differed substantially between aphid species, such as GAA and RAA, but also RAA and BCA. Obviously, as with any ecologically sound intervention, the technique needs to fit the ecosystem and not the other way around (Tittonell, 2014). Indeed, aphid species themselves may further shape the relative importance of natural enemies. Although in this study both species of aphids declined, GAA harbored substantially lower numbers of the three key natural enemies, which may indicate that other natural enemies or mechanisms, not monitored here, contributed to their decline. Owing to differences in intrinsic growth rate particularly at lower temperatures (Graf et al. 1985), GAA may thus be comparatively more targeted by other, freely ranging generalist predators, which were not quantified in this study. The fact that application of Ant Stop in cherry did not suppress BCA (*A. cerasi*) to the extent as RAA, also underlines that tailoring of solutions need tailoring to the agroecosystem. More detailed studies are needed on this point.

Finally, our results over four seasons of intervention illustrate that timing is critical for impact. In 2019 as well as 2022, the critical window for effectively controlling *D. plantaginea* was missed. Given the explosive growth of RAA colonies, the emergence of foundatrixes need to be more rigorously coupled to the phenology of the crop and seasonal temperature data. Further research is needed to make predictive models with which farmers can time future applications of Ant Stop.

Conclusions

This study demonstrates an important proof-of-concept of the sustainable control of the rosy apple aphid through diverting ants using artificial honeydew (Ant-Stop) at orchard scale in Swedish apple orchards. This method would appear entirely sustainable, and in fact rebuilds ecosystem resilience by augmenting natural enemy populations in the process. That being said, further research on augmentation of control using attractions, timing of application, tailoring Ant Stop to aphid pests in other crops, and formulation issues are among the challenges that need further research (see also relevance and recommendations).

Relevance and recommendations

The results are highly relevant for the apple growers in Sweden as well as in the rest of Europe, as RAA is considered THE main pest of apple in Europe. The ability control aphids through diverting ants at large scale and the synergistic effects of using lures, together with the large market, offers good perspectives on a commercial interest for both products and thus ultimately availability to growers.

Main questions that remain after our research are:

1. The final **formulation** which farmers can apply. SPLAT did not sufficiently work as bait for ants, while sugar vials cannot be applied in an orchard. We currently work on **alginate/gelatin/agar formulations** which will be the focus of continued research in

2023/2024. We are hopeful that this entirely sustainable solution can be formulated as a sprayable prophylactic formulation in the near future.

2. **Timing** of application (and longevity of Ant-Stop formulation): in the course of the experiments over the years, we noticed very substantial differences in RAA control in apple. In each case were suppression we insufficient, application the artificial honeydew started too late. Different from GAA's, RAA colonies show a strongly exponential growth in the early season, which can be missed. The intervention my still reduce tending aphids by ants, but the natural enemies are still suppressed by the few ants present in the colonies. It thus appears critical to apply the artificial honeydew preventatively, as it does not work curatively. **The exact window of application needs to be further investigated** possibly using day-temperature data, such that farmers can anticipate and prevent RAA outbreak.
3. **Duration of the diversion** (Ant-Stop). Inasmuch as timing of the start of application needs further fine-tuning, the end of application needs further investigation too. We noticed that application of artificial honeydew had an immediate effect on the number of ants in the tree and tending aphids, thus breaking myrmecophily. Since RAA shows an exponential growth in the early season, the effects on colony establishment and growth is immediate. This raises the question **how long artificial honeydew needs to be present to disrupt RAA colony growth and circumvent pest buildup**. Preliminary results from 2022 indicating that 2-3 week intervention may suffice. This will be further tested in the coming season. Naturally this parameter has important consequences for the **formulation and for application strategies**. Also, minimizing intervention may be favorable to prevent any side effects of ant diversion. Although no such side effect has been observed yet, ants are important predators on other pests in the orchard and thus contribute to ecosystem services.
4. The effect of ant diversion on RAA development was planned to **be tested in other climate zones**, notably in France, Belgium and Poland. In part due to covid and being dependent on collaborations, this has unfortunately not materialized yet. It goes without saying that **this remains to be done**. Based on several independent studies, we are confident that ant diversion will suppress RAA also in other climate zones. This would increase the market potential, and thus make a further development of a product worth the while. We hope that through collaborations we will still be able to test ant diversion in other parts of Europe.
5. The **strong fluctuations in the relative importance of resident predators** in aphid colonies between orchard types and across years, raises questions about whether a particular **lure for a certain natural enemy will be effective across years**. In addition, the relative impacts of each of these resident predators (along with transient predators, which were not scored) need to be more firmly established under different circumstance to assure that across conditions baits for syrphid flies and lacewings will indeed have the desired increase in biocontrol which were observed here.
6. While the **formulation of these lures** appears to be fine, we do not have a good grip yet on their **field life** (is one application sufficient?), and whether formulations can be combined into one and attract multiple natural enemy guilds (such as both lacewings and syrphid flies). Finally, optimization of the baits, particularly for syrphid flies, would appear important to secure efficacy.
7. Ants dominate virtually any aphid pest system, and thus the question is whether this **innovation can be propelled into other crops**, including annuals. Using a small seed fund, we are currently investigating the possibilities of aphid control in sugar beet. Proof of principle exists in barley (see publications).
8. While ant diversion worked similarly well for the green apple aphid (GAA, *Aphis pomi*), it is not considered a pest. However, the **wooly apple aphid** (WAA, *Eriosoma lanigerum*), is another important apple pest. Yet, as it is an irregularly pest in Southern Swedish apple production we have not been able to test control of this species using ant diversion. **Further tests, likely outside Sweden are needed**.

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Result dissemination:

State all result dissemination from the financed project into the appropriate section below, including information as indicated. Additional rows can be added to the table.

Scientific publications, *published*	Pålsson J., Porcel M., Frimodt Hansen M., Offenberg J., Nardin, Larcher R. & Tasin M.. 2020. Aphid-infested beans divert ant attendance from the rosy apple aphid in apple-bean intercropping Scientific Reports 10, 8209. Doi: 10.1038/s41598-020-64973-7
	Badra Z., Larsson Herrera S., Cappellin L., Biasioli F., Dekker T., Angeli S. & Tasin M. 2021. Species-Specific induction of plant volatiles by two aphid species in apple: real time measurement of plant emission and attraction of lacewings in the wind tunnel. Journal of Chemical Ecology, 47, 653–663. Doi: 10.1007/s10886-021-01288-5
Scientific publications, *submitted*	Larsson Herrera S., Badra Z., Frimodt Hansen M., Chakravarthy Shankarkumar A., Kleman I., Tasin M., Dekker T. Ecological intensification for biocontrol of aphids requires severing myrmecophily.

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Scientific publications, *manuscript*	Larsson Herrera S., Tasin M., Dekker T. Lures for natural enemies synergize diversion of ants in biocontrol of rosy apple aphids in organic and IPM apple orchards.
Conference publications/ presentations	*Due to covid and travel restrictions, the work has been presented in a limited number of conferences and workshops*
	Agroecological Plant Protection Through Habitat Manipulation, 2nd Agroecology EU Forum (conference), Heraklion Crete, September 2019.
	Innovative ecosystem management for sustainable pest control: controlling aphids through manipulating ants and natural enemies. Trädgårdkonferensen, Lund, March 2020. Co-organized the meeting
	Toward a pesticide free orchard. Online workshop, API-tree, September 2021. Co-organized the meeting.
	Ecological intensification for biocontrol of aphids requires severing myrmecophily. Växtskyddskonferens, Uppsala, November 2022.
	Control of rosy apple aphids without insecticides through: diversion of commensal organisms. "Forskning för framtidens ekologiska production" (conference), Uppsala, Dec 1 2022.
Other publications, *media etc.*	
	Ellström A., Socker till myror stoppar bladlössen. Land Lantbruk 3-15 Jan. 2021.
	Hansson, E., Kan bladlössens vakter luras bort med socker? Natursidan.se, Nov. 2022
Oral communication, *to sector, students etc.*	*Due to covid and travel restrictions, the work has been presented in a limited number of conferences and workshops*
	Bekämpning av äpplebladlöss genom lokbeter för myror. LRF medlemsmöte om fruktproduktion. Höör, Mars 2020.
	Designing diverse orchard systems, 1st API-tree workshop February 2nd, 2018. Organizer. An online EU-workshop for researchs, advisors and growers, Sept 2021.
	Kontroll av bladlöss i sockerbetor och äpplen genom distraktion (av myror), attraktion och belöning (till naturliga fiender). Möte Växtskyddsrådet, Sept 2020.
	In addition, the topic is being presented annually to agroecology students, and has been presented regularly in other contexts such as in seminars at other universities (e.g. Makerere University, Uganda, Nov 2021, MUCE, Iringa, Tanzania, Jan 2023
Student theses	Larsson Herrera S. 2022. Data in, lures out: designing selective lures against fruit pests. PhD thesis. Supervisor, Teun Dekker, Marco

	Tasin, https://pub.epsilon.slu.se/27918/1/larsson-herrera-s-220517.pdf
	Chakravarthy A. 2021. Assessing the growth and development of the rosy apple aphid (Dysaphis plantaginea) by altering ant-aphid mutualism. Project work. Supervisor: Teun Dekker
	Yang H. 2023. Rosy apple aphids' control with artificial honeydew in an organic apple orchard in Sweden. MSc thesis. Supervisor: Teun Dekker.
Other including Patents	*Patents, book chapters, reference group meetings etc.*

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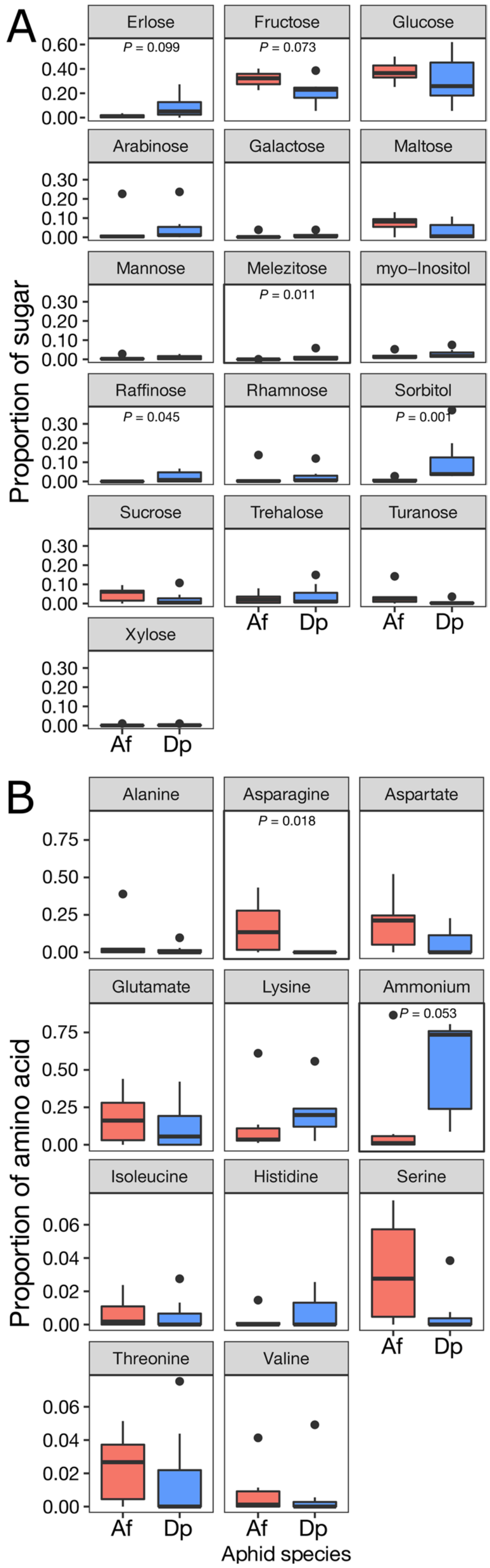

Fig. S1. Composition of honeydew of the bean aphid, *Aphis fabae*, and the rosy apple aphid, *D. plantagineae*. Each of the different components of sugar and amino acids are expressed as a proportion of the total content in honeydew. Note that in spite of the low amounts of melizitose, the trisaccharide was highly preferred by *L. niger* in preference assays.

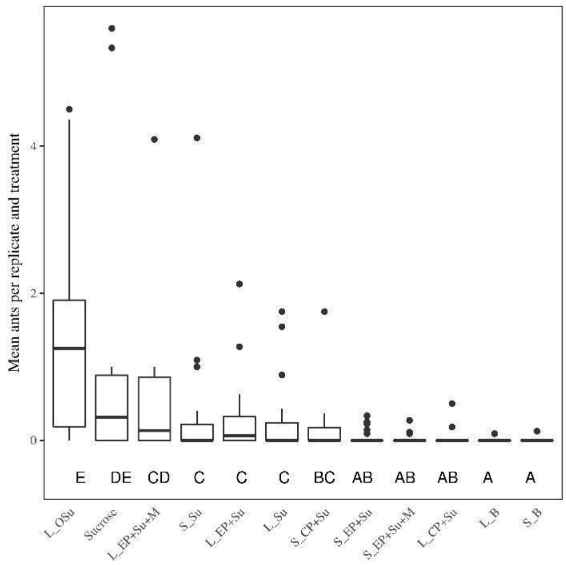

Fig. S2. Attractiveness of various ant baits formulated as a base solution, or with a SPLAT base. L = liquid solution, S = SPLAT as carrier. OSu = original sugar composition, EP = egg protein, CP = corn protein, Su=sucrose, M= melizitose, B=base.

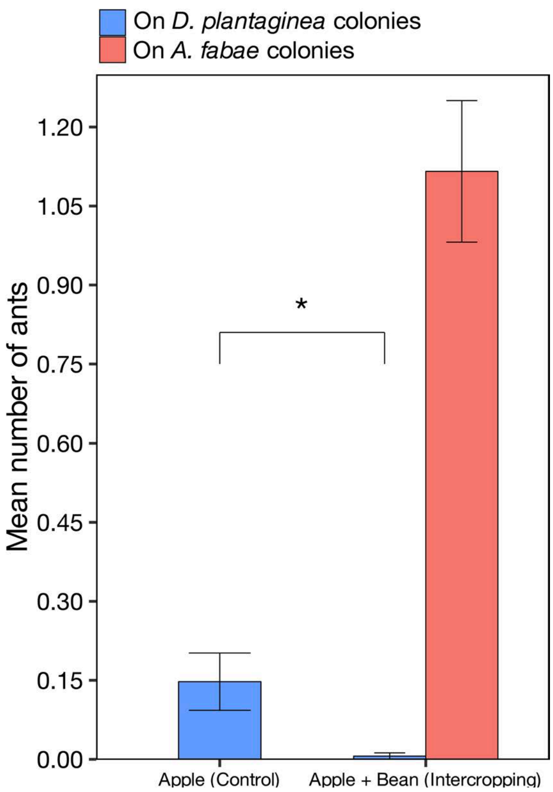

Fig. S3. Diversion of ants using bean intercropping. Bean plants were germinated and potted and infested with *A. fabae* in the greenhouse prior to the experiments. Infested plants were placed in the field next to *D. plantaginea* infested apple trees and ant tending frequencies noted. Companion bean plants next to apple tree next to eliminated ant tending of *D. plantaginea* colonies. The use of companion bean plants proved however unpractical under field conditions, given the difference in phenology of beans and apple and their aphids. In addition, companion plants are difficult to scale at orchard level.

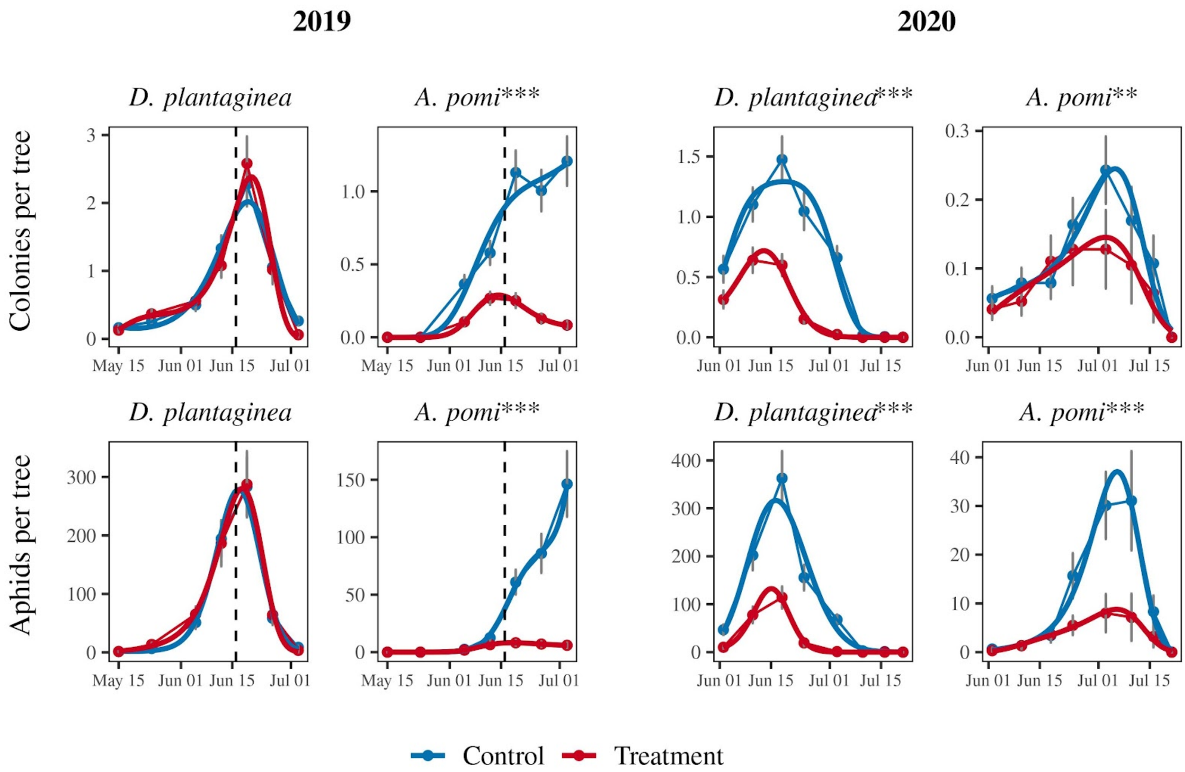

Fig. S4. Comparison of timing of intervention on the success of control. In 2019, the intervention was applied after the early exponential growth phase of RAA colonies, which led to a failure of control. While 2021 resembled 2020, in 2022 the intervention window was missed again, and application was too late, leading to similar results as in 2019. 'Prophylactic' application of Ant-stop is critical to control of RAA.

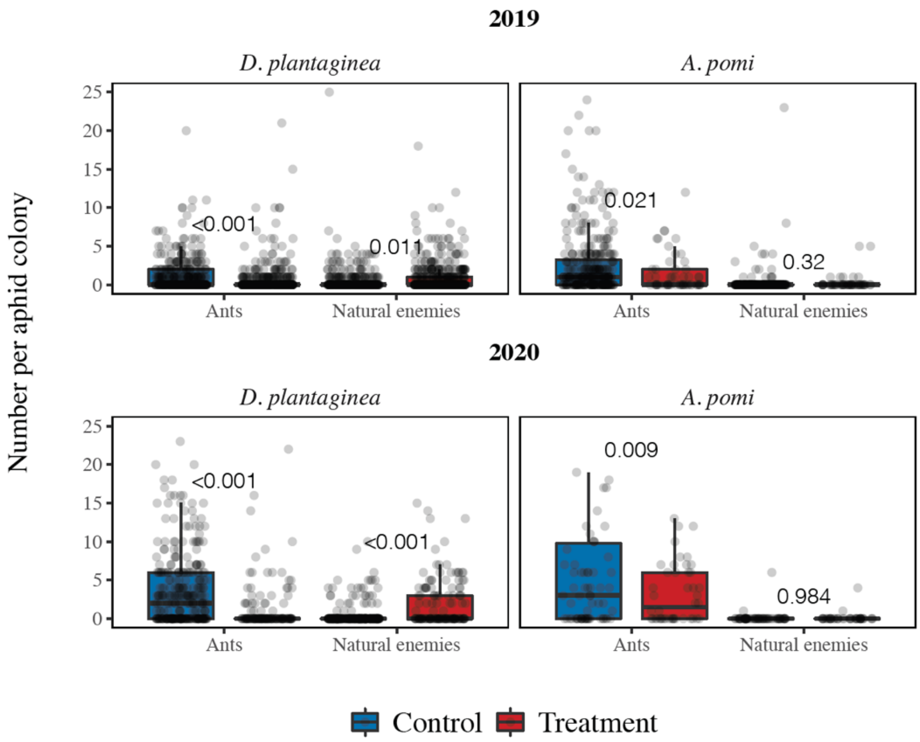

Fig S5. Total number of ants and natural enemies in aphid colonies in 2019 and 2020 (after placement of artificial honeydew). The p-value (above each set of box plots) is the result of a generalized linear mixed model fitted with a negative binomial distribution using tree as the random effect.

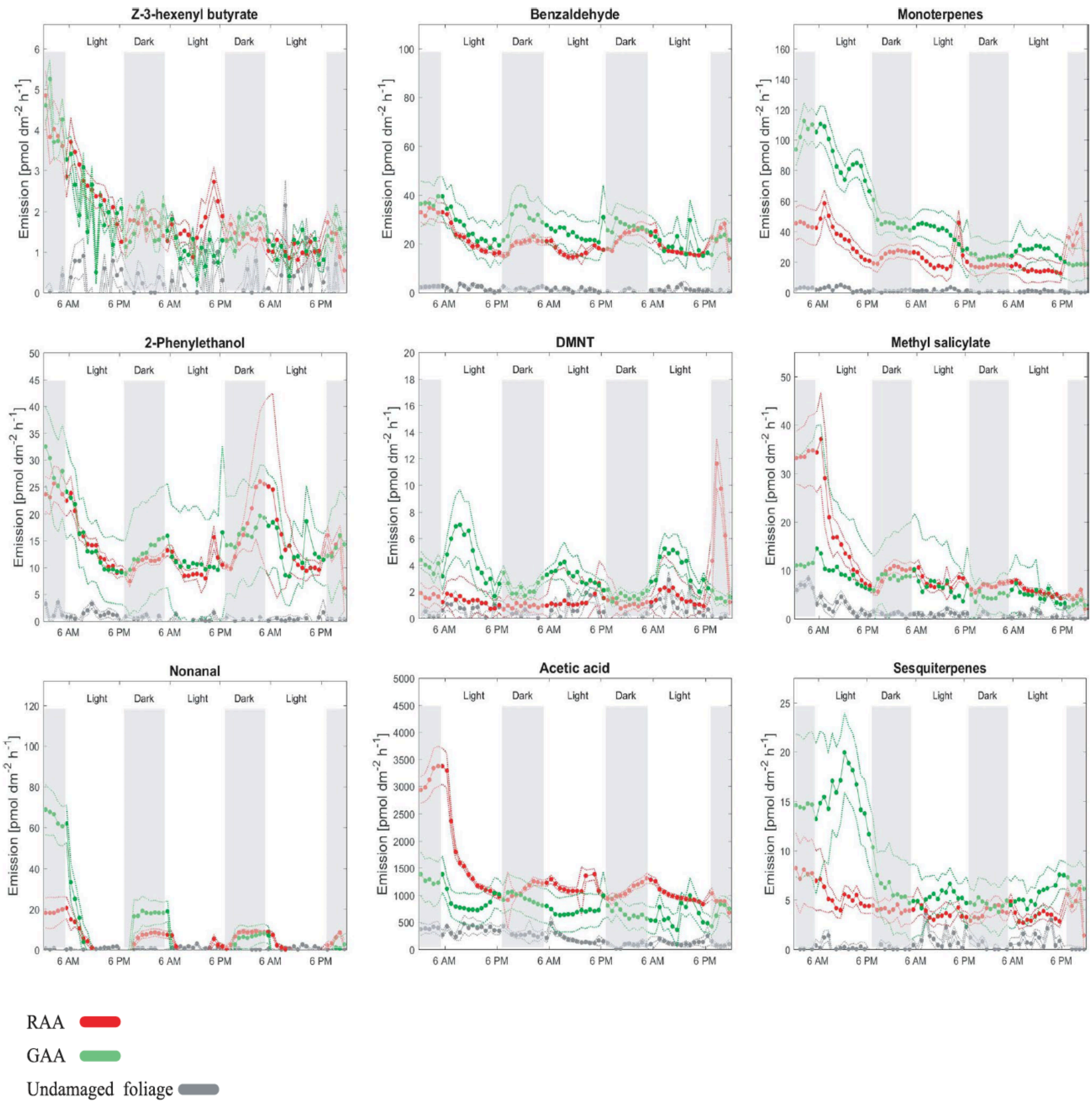

Fig. S6. Emission rate (pmol dm−2 h−1) of volatiles detected by PTRToF–MS in the headspace of undamaged apple trees (in red), infested with green apple aphid (in green), rosy apple aphid (in grey) over a period of three days. Plants were infested with aphids 72 h prior to the first measurements. White and grey-shaded areas indicate day/night cycle (14 h photoperiod)