Plant resistance ecology – the role of resistance in plant-insect mutualisms

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Cover: “Let me take you down, ‘cause I’m going to Strawberry fields”
(The Beatles: Strawberry Fields), Drawing by Daniela Weber

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Abstract

Global food security is largely dependent on the use of synthetic pesticides for pest control. This extensive reliance on pesticides, however, has promoted widespread loss of insect diversity; thereby jeopardizing the provision of insect ecosystem services like pollination and biological control. For future sustainability, agricultural practices need to shift from pesticide-dependency towards more balanced approaches such as integrated pest management (IPM).

Strawberry cultivation is in particular highly pesticide dependent. To protect pollinators, pesticides could be avoided, but reduced pesticide use might promote floral damage by herbivores and lower yields. Indeed, results from this thesis show that in woodland strawberry (*Fragaria vesca*, L.) floral damage by strawberry leaf beetles (*Galerucella* spp.) had indirect effects on pollination and direct effects on yield. Pest-damaged flowers yielded smaller fruits, even when pollinated by hand, and were avoided by pollinators. Breeding crop cultivars for pest resistance is a corner stone of IPM and could provide a potential solution to prevent pollination deficits in low-pesticide cultivation.

Crop wild relatives like woodland strawberry are predicted to show high heritable variation in resistance traits useful for crop improvement. Here, wild accessions of woodland strawberry from Sweden were screened for resistance against strawberry leaf beetles. Heritable genetic variation was found for each plant resistance indicator and oviposition was avoided on resistant plant genotypes. Thus wild woodland strawberry can be deemed to offer a promising resource for restoring pest resistance in cultivated strawberry.

However, modifying plant resistance could also affect the herbivore's natural enemies. Parasitoid performance was in fact strongly affected by herbivore diet source when tested with different wild woodland genotypes. Yet genotype resistance against the parasitoid's host, the strawberry leaf beetle, did not predict parasitoid survival. Rather, parasitoid survival was explained by plant foliar chemistry, of which levels of carbohydrates appeared to be the most important for parasitism success. These findings underline the need to assess plant-quality effects on biocontrol in plant breeding programs for a synergistic application in IPM.

Taken together, this work demonstrates that deeper insight into trophic interactions between crop plants, pests, and mutualists (e.g. pollinators and natural enemies) will be crucial in order to design optimal IPM strategies which suppress pests and support mutualists.

Keywords: biological control, crop wild relative, florivory, *Fragaria vesca*, *Galerucella*, integrated pest management, metabolomic profiling, pollination, tritrophic interactions

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Dedication

To Mintha and the less furry members of my family

Crivens!

Battle cry of the Nac Mac Feegels, from The Wee Free Man by Terry Pratchett

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Muola A, Weber D, Malm LE, Egan PA, Glinwood R, Parachnowitsch AL, Stenberg JA (2017) Direct and pollinator-mediated effects of herbivory on strawberry and the potential for improved resistance. Frontiers in Plant Science 8:823.

II Weber, D., Egan, P. A., Muola, A., and Stenberg, J. A. (2019). Genetic variation in herbivore resistance within a strawberry crop wild relative (*Fragaria vesca* L.) (submitted)

III Weber, D., Egan, P. A., Muola, A., Ericson, L. E. and Stenberg, J. A. (2019). Plant resistance does not compromise parasitoid-based biocontrol of a strawberry pest. (manuscript)

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The contribution of Daniela Weber to the papers included in this thesis was as follows:

I Planned and performed part of the experiments. Participated in the writing of the paper which was led by A. Muola.

II Collected and reared plant and insect material. Planned and performed the bioassays. Analysed the data and wrote the manuscript with the input of the co-authors

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1 Background

Pest insects have reduced the productivity of crops since the dawn of agriculture. Cultivating many individuals of the same plant species in a large agricultural field is an excellent breeding ground for herbivorous insects to build up a large population (Snyder and Ives, 2009). Humans have been looking for ways of safeguarding crops and harvest ever since and still global food security relies heavily on pest control. Yet even in spite of present-day protection measures, insect pests are still responsible for an average of 16-18% loss of agricultural yields worldwide (Oerke, 2006; Oerke and Dehne, 2004).

Herbivorous pests can compromise yield either through direct (i.e. physiological) damage to the plant, but also indirectly through influencing ecological interactions with mutualists such as pollinators (Hladun and Adler, 2009; McCall and Irwin, 2006; Strauss, 1997). Little is known about the impact of pollination success on economically valuable fruit traits like appearance, flavour, shelf life, and harvest timing, and even less is known about the extent to which herbivores can mediate pollination success. For pollinated crops like fruits and vegetables, insufficient pollination can lead to lower and less consistent yields, deformed fruits, or less flavor and quality (Klatt et al., 2013; Nicholson and Ricketts, 2019).

In commercial strawberry (*Fragaria x ananassa*, Duchenes), for example, annual yields would be reduced by about 45 % with no pollination (Hodgkiss et al., 2019), and pollinators contributed 39% to a total of 2.90 billion US$ made from selling 1.5 million tons of strawberries in the EU in 2009 (Klatt et al., 2013). Maintaining effective insect pollination service is critical to maintain high quality harvest, therefore more knowledge on how herbivores can mediate pollination is needed to minimize pest damage while maximizing pollination.

1.1 Pesticides: from friend to public enemy

During the last century, plant protection products became an important component of agricultural systems around the world. Starting in the 1940s, synthetic plant protection chemicals – so called pesticides (e.g. herbicides, insecticides, fungicides) – became available on the global market (Carvalho, 2017). This occurrence marked the starting point of a far-reaching agricultural intensification process leading to higher yields, but also to a profound simplification of the agricultural landscape (reduction of crop rotation, crop diversity, and of natural habitats) (Stoate et al., 2001; Tscharntke et al., 2005). The application of synthetic fertilizers and pesticides rapidly replaced many biological processes involved in crop production such as nutrient recycling or control of insects (Bommarco et al., 2013; Tscharntke et al., 2005). Consumers became accustomed to impeccable fruits and vegetables and everything blemished became more or less unsellable on the fresh market (Sponsler et al., 2019). With such high dependency on external inputs to maintain productivity and expected quality, the yearly global production of pesticides rose from 0.2 million tons in 1950s to more than 5 million tons by 2000 (Carvalho, 2017; FAO, 2017).

Yet, the achievement of high agricultural productivity also came at a high cost to the environment and biodiversity. Not long after the large-scale introduction of pesticides, negative side-effects on human health and environment became apparent (Bommarco et al., 2011; Carson, 1962). Especially the first synthetic and broad-spectrum insecticides, like DDT, caused serious, far-reaching damage to human health, biodiversity and ecosystems. Soon their overuse eroded natural pest regulation and led to pest resurgence, and secondary pests (Bommarco et al., 2011, 2013; Carson, 1962; Carvalho, 2017). Starting with the first public debates in the early 1960s, the increased awareness and concern about the risks of synthetic pesticides among the public and scientific community has led to significant changes in agricultural policies and regulatory restrictions of pesticides to reduce harm to the environment (Carvalho, 2017; Pimentel, 2005; Stoate et al., 2001). Formulation and applcation technics of pesticides have subsequently been made more specific to the targeted pest to minimize side-effects (Peterson et al., 2016; Pimentel, 2005; Stoate et al., 2001).

Resistance to pesticides continues to steadily evolve among pest species and is now so widespread that it is even listed as the third most serious threat to global agriculture (Peterson et al., 2016; Pimentel, 2005; Stoate et al., 2001). In parallel, pesticides have repeatedly been demonstrated to be lethal or sublethal to a wide range of beneficial arthropods such as pollinators and natural enemies; thereby undermining pollination and natural pest control services to crops (Bommarco et al., 2011; Desneux et al., 2006). Even with the development of new generation pesticides, negative side-effects and exposure to non-target organisms and the ecosystem still remain inevitable (Mullin, 2015; Sponsler et al., 2019). Most recently, the European Union has issued a ban on several neonicotinoid insecticides for outdoor use (European Commission, 2018a, 2018c, 2018b) due to their negative impact on beneficial arthropods and unacceptable risk to pollinators (European Food Safety Authority, 2018).

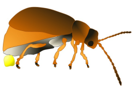
Pollinators, wild as well as managed, facilitate pollination and fertilisation in approximately 88% of all flowering plants (Sponsler et al., 2019). Most of the fruit, vegetable, and nut crops that enrich today's human diet with important nutrients such as vitamins rely on pollination by insects (Isaacs et al., 2017). The evident decline of insect pollinator populations and high overwintering mortality of honey bee colonies across Europe and North America has caused serious concern not only among ecologists and agricultural experts but also the public at large (Ollerton, 2017; Potts et al., 2010; Seitz et al., 2015). Besides habitat loss and landscape simplification, pesticides are suspected to be one of the crucial factors causing not only the pollinator decline but also a far reaching general loss of biodiversity (Bommarco et al., 2013; Sponsler et al., 2019; Stoate et al., 2001). This loss in biodiversity concerns also natural enemies and many species, especially specialists like parasitoids, show reduced abundances or are threatened by local extinctions (Jonsson et al., 2012; Lamichhane et al., 2016).

Given that pollinator declines are nested within larger patterns of arthropod decline and biodiversity loss, the debate about banning pesticides and implementing more sustainable and environmentally friendly modes of agriculture has become even more urgent.

1.2 Wanted: alternative for pesticides

Mounting evidence of the importance of beneficial arthropods, and of their decline worldwide, has prompted efforts to conserve and restore them within agricultural landscapes. Within these landscapes, non-crop habitat plays an important role in regulating and supporting vital ecosystem services such as pollination and biological control of agricultural pests; a point which is also increasingly acknowledged by policy makers (Bommarco et al., 2013).

Over the past years EU policy has been directed towards achieving significant reductions in pesticide use, as well as encouraging the transition from conventional crop protection systems towards a sustainable agriculture (Barzman et al., 2015; Hillocks, 2012; Lamichhane, 2017). This fast-growing demand for alternative, non-chemical ways for pest management has thus reignited interest in the concept of integrated pest management (IPM). IPM



decision making takes into consideration the biology, ecology, and economics of the crop and targeted pest, and combines together a diversified suite of compatible control tactics (mechanical, physical, cultural, chemical, biological, and host plant resistance). In this way, IPM can provide economically viable pest control while minimizing hazard to humans, beneficial organisms, and the environment (Kogan, 1998; Radcliffe et al., 2009). Although promoted since the 1970s as a way to reduce pesticide use, the implementation of IPM principles has been hastened in past years by the European directive on the sustainable use of pesticides (Barzman et al., 2015; Directive 2009/128/EC, 2009). Although this momentum offers a unique opportunity to advance sustainable agriculture, there are still challenges related to IPM adoption which need to be addressed before implementation can become commonplace (Lamichhane et al., 2016).

1.2.1 Let's get wild – plant resistance in IPM

The crop itself provides both a target and a foundation for integrated pest management (IPM). The use of herbivore-resistant plant cultivars is thus one of the key techniques and a promising option to gain independence from heavy pesticide use during cultivation (Broekgaarden et al., 2011).

Given the wide availability of insecticides, breeding for insect resistant cultivars has been a relatively low priority, and plant resistance has come under serious investigation only in the last 20 years (Rodriguez-Saona et al., 2019). Focused on high productivity and easier cultivation, crop cultivars have been bred for a simpler morphology, photoperiod insensitivity, more exaggerated physical traits like bigger fruits, altered nutritional content, and reduction or loss of toxic compounds (Chen et al., 2015b, 2015a). Successive rounds of artificial selection and domestication yielded the crops that feed the world today, but have also resulted in a distinct loss of genetic variation among modern crop cultivars. As a consequence, potentially valuable genetic variants and associated phenotypes have been filtered out of crop germplasm, leaving a very narrow genetic base and low adaptive capacity within today's agricultural systems (Broekgaarden et al., 2011; Chen et al., 2015a; Dempewolf et al., 2017; Liston et al., 2014).

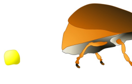
A good source to widen the genetic base of crops are their related wild species and progenitors; so-called crop wild relatives. Uninfluenced by human driven selection, crop wild relatives have adapted to a wide range of habitats. Habitat and genetic diversity complement each other and crop wild relatives have the potential to harbour a substantial amount of genetic diversity (Chen et al., 2015a; Fiedler et al., 2008; Seiler et al., 2017). From this genetic diversity new allelic variation for most traits required in breeding programs can be drawn. Commonly sought-after traits include those which enhance and restore crop resistance to pests and disease, but also traits underpinning abiotic stress, yield, and quality (Egan et al., 2018; Hajjar and Hodgkin, 2007; Seiler et al., 2017; Vincent et al., 2013).

Known as ‘rewilding’ or ‘inverse breeding’ crop wild relatives can be used in breeding programs to re-establish plant traits lost or degraded during crop domestication (Andersen et al., 2015; Dempewolf et al., 2017; Egan et al., 2018; Palmgren et al., 2015). Over the past few decades, the incorporation of wild crop relatives in crop improvement programmes has grown steadily. There have been significant successes such as the introduction of late blight resistance from the wild potato (*Solanum demissum* Lindl.) (Prescott-Allen and Prescott-Allen, 1986), and cabbage root fly resistance from wild white mustard (*Sinapis alba* L.) into oilseed rape (*Brassica napus* L.) and rutabaga (*B. napus*. var. *napobrassica*) (Ekuere et al., 2005; Malchev et al., 2010). The ‘rewilding’ of cultivars is predicted to further increase especially in pesticide intensive crops such as strawberries (*Fragaria x ananassa*) in order to meet the demand to increase agricultural sustainability whilst simultaneously maintaining productivity (Hajjar and Hodgkin, 2007; Lane and Jarvis, 2007; Vincent et al., 2013). Thus further studies to investigate genetic variation in natural population of wild crop relatives are needed to lay the groundwork for potential future ‘rewilding’.

1.2.2 The foe of my enemy is my friend

Besides breeding for and using resistance cultivars, biological control is another key approach in the concept of IPM (Eilenberg et al., 2001). Each herbivore species has a number of natural enemies, ranging from birds over predatory arthropods, parasitoids, to pathogens. Natural enemies often regulate pest population growth in agricultural fields providing a crucial ecosystem service: the top-down control of agricultural pests (Bommarco et al., 2013).

Biological control programmes seek to use this ecosystem service to their advantage, and employ enemies (biological control agents) of the targeted pest to control the pest and to keep population density below the economic threshold (Bale et al., 2008; Eilenberg et al., 2001). This principle has been part of agricultural production for centuries, where for instance cats have been used to control rodents for thousands of years (Caltagirone, 1981). One of the first modern scientific approaches dates back to the late 19th century, to the controlled release of an insect predator to control for an invasive scale insect devastating the citrus cultivation in California at that time (Bale et al., 2008; Caltagirone, 1981). Within modern biological control programmes four separate strategies can be differentiated (Eilenberg et al., 2001):



- *Classical biological control* deals with the control of invasive pests and is the introduction and establishment of one or more biological control agents, with the intention of long-term pest control.
- *Inundation biological control* is the short term mass release of biological control agents to achieve a knock-down effect on pest population that has reached the economic threshold.
- *Inoculation biological control* is the release of biological control agents over a definite period to control a pest for a longer time but not permanently and is often practiced during growing season in controlled environments such as greenhouses
- *Conservation biological control* involves modification of the environment in the agroecosystem and/or agronomic practices to support and enhance natural enemy populations already present in the agroecosystem to reduce pest attacks.

Among those four strategies, only conservation biological control is independent from rearing and releasing natural enemies. Arthropod natural enemies can dwell in all different kinds of cropping systems and their presence can go largely unnoticed until populations are diminished or removed (e.g. pesticide application) resulting in pest outbreak or re-occurrence (Naranjo et al., 2015). In the late 1970s, for example, naturally occurring predators, and in particular spiders, were removed from rice paddy by the overuse of broad-spectrum insecticides leading to severe outbreaks of the brown plant hopper (*Nilaparvata lugens*, Stål) throughout Southeast Asia (Kogan, 1998). Interest in conservation biological control has increased substantially over the past two decades, since the maintenance of natural enemy populations can provide practical and sustainable pest control, especially for high- acreage annual field crops and crops that depend on pollinators (Fiedler et al., 2008; Jonsson et al., 2008).

1.2.3 You are what you eat: a trophic conflict becomes apparent

Within IPM several control methods are typically combined to reduce pest damage. As a consequence the two of the key approaches, plant resistance and biological control, are often implemented at the same time. However, whether both additively contribute to reduce pest levels depends on the mechanisms involved in plant resistance and the effects they have on natural enemies. Plants typically employ multiple lines of defence against insect herbivores, and crops have been bred for various chemical and physical plant traits to enhance their capacity to directly resist pests.

Physical traits such as leaf toughness or trichome density can significantly decrease the feeding efficiency of herbivores. However natural enemy movement can be disrupted as well, which in turn can significantly decreases consumption rate of the natural enemies (Cortesero et al., 2000; Peterson et al., 2016). A higher trichome density for example, typically lowers the attack rate of natural enemies since dense trichomes physically hinder foraging (Riddick and Simmons, 2014). The increased slipperiness of the leaf surface, on the other hand, can frequently cause natural enemies to fall off leaves, substantially decreasing their search and attack efficiency (Grevstad and Klepetka, 1992).

Direct chemical plant defence traits are most commonly used in crop breeding and have been selected in crops almost exclusively in a bitrophic context excluding natural enemies. Yet, rendering the plant tissue as nutritionally unsuitable, deterrent, or toxic to the herbivore may also decrease the quality of herbivores as prey or host for natural enemies (Harvey, 2005; Pappas et al., 2017; Peterson et al., 2016). Herbivore quality is fundamental for the developmental and reproductive performance of natural enemies and to permit a sufficient natural enemy population build-up (Hunter, 2003).

Amongst the natural enemies most frequently used in biological control programmes, parasitoids are especially sensitive to quality changes in the herbivore’s food plant (Bukovinszky et al., 2012; Harvey and Gols, 2018; Pappas et al., 2017; Santolamazza-Carbone et al., 2014). Parasitoids are insects that are free-living as adults and parasitic during their larval stage. The diverse larval development strategies of parasitoids are closely attuned with that of the host, and range from living outside a paralyzed host to developing inside the host body. Unlike insect predators, which consume many different prey items to fulfil their nutritional needs, parasitoid offspring are confined to the limited resources contained within a single herbivore host (Bukovinszky et al., 2012; Harvey, 2005; Harvey and Gols, 2018). There is clear evidence that the quality of the herbivore’s diet influences the herbivore’s haemolymph composition and as such its nutritional suitability for the parasitoid, and the ability of the herbivore’s immune system to fight off parasitism (Harvey, 2007; Kaplan et al., 2016; Ode, 2006; Singer et al., 2014; Thompson, 2005; van Geem et al., 2016). Taken together, negative effects on parasitoid fitness can occur via:

- changes in the host herbivore’s vigour, including immunological responses
- insufficient resource availability for the parasitoid offspring
- plant toxins or defence compounds that reach the haemolymph unmetabolized; where they are directly encountered by the parasitoid offspring
- plant toxins or defence compounds that are actively sequestered by the herbivore host as defence against parasitism

The extent to which chemical plant defences are deflected unto the parasitoids depends among other things on the herbivore’s capability and investment in detoxification, excretion, or sequestration of the plant defence compounds (Hunter, 2003; Kaplan et al., 2016; Pappas et al., 2017).

Given the wide variety and function of chemical compounds within plant tissues, host plant dietary quality in a tritrophic context is hence a complex property (Hunter, 2003; Pappas et al., 2017). More than 100,000 secondary plant compounds have been identified so far and many contribute to plant herbivore defence (Agrawal, 2011). Furthermore, plant tissues provide essential nutrients (e.g. proteins, sugars, minerals) which strongly affect the feeding efficiency, developmental, and reproductive performance of herbivores (Mason et al., 2014; Peterson et al., 2016; Thompson, 2005). So far, the focus of much research has been limited to the tritrophic role of toxic secondary plant compounds. Plant compounds that are not obvious defence compounds have been paid less attention, despite their potential importance in influencing herbivores’ vigour (Ode, 2006; Soufbaf et al., 2012).

Natural enemies can be considered an extension of plant defence by attacking the insect herbivores that feed on plant tissue. Acting as indirect defences, many plant species are known to release herbivore-induced plant volatiles (HIPVs) to attract parasitoids and other natural enemies facilitating the location of the herbivore. Providing a volatile cue may backfire when it leads to poisoning or impairing instead of conserving the natural enemy due to increased direct plant defences. The integration of plant resistance traits with biological control therefore needs careful consideration.

Evaluation of the joint effects of herbivore-resistant crops and biological control programs on pest population dynamics is essential to foster effective and sustainable pest control. Furthermore, evaluating and developing novel crop varieties from a biocontrol perspective can help to decelerate or prevent herbivore adaptation to plant resistance (Peterson et al., 2016; Raymond et al., 2007; Stenberg, 2017). Natural enemies can delay the development of such resistance in a pest population if the natural enemies favour resistant pest individuals and add to fitness costs associated with resistance to crop traits (Raymond et al., 2007). But alternatively natural enemies may aid selection for resistance if susceptible pest individuals are attacked more frequently (Gould et al., 1991). Yet, in the process of breeding and assessing resistant crop varieties, the potential impacts on natural enemy performance is scarcely considered (Jonsson et al., 2008; Peterson et al., 2016; Schädler et al., 2010). Thus, little is known about the extent of potential (in)compatibilities of biological control and current plant breeding programs for resistance.

2 Aims

Plants form the main link between insect herbivores and beneficial arthropods such as the plant's pollinators and the herbivore's natural enemies. Modifying plant resistance against herbivores, therefore, might not only influence the herbivore, but also the ecosystem services of pollination and biological control of agricultural pests. Nowadays crop production is shifting from heavy pesticide-dependency towards IPM, and in order to implement IPM successfully a good understanding of different trophic interactions is crucial. The aims of the thesis were therefore the following:

1) To explore the effects of herbivory on pollinator behaviour, pollination success, and the consequences for yield and fruit appearance in woodland strawberry

A 'no pesticides' policy during or prior to crop flowering is often implemented to avoid direct pesticide exposure to pollinators. Such a practise, however, might lead to increased herbivory during flowering and a higher risk of herbivore damage to the flowers. Depending on the type of herbivore damage, the flowers might still be functional, but little is known about the impact of partial flower damage by herbivores on pollination services and subsequently on overall yield and economically valuable fruit traits such as fruit appearance.

2) **To screen natural populations of the woodland strawberry for variation in resistance against a herbivorous pest insect in strawberry**.

With the promotion of IPM, resistant plant varieties have reclaimed their role as an important component in insect pest control, and the utilization of crop wild relatives in crop improvement programmes has grown steadily. However, before exploitation of genetic resources can in any way take place natural populations need to be sampled, conserved, and screened for potential variation in valuable traits like herbivore resistance. In order to facilitate a robust basis for herbivore resistance both the components of herbivore performance and behaviour should be taken into account.

3) To explore the effects of plant resistance on parasitoid fitness and to relate the parasitoid survival to both secondary and primary plant compounds

In IPM, herbivore resistant crops are commonly used together with biological control. Plant-mediated variation in herbivore quality is important for parasitoid fitness and performance, but still little is known about the potential synergistic, complementary, or disruptive interactions when both control methods are used together. Similarly little is known about the joint effects of primary and secondary plant compounds on parasitoid performance, since most previous work has focused on a limited set of secondary plant compounds.

3 The Study System

3.1 The Woodland Strawberry – a Wild Relative of the Garden Strawberry

Figure 1. Wild woodland strawberry, *Fragaria vesca*, L. in bloom. Photo: Paul Egan

The woodland strawberry *Fragaria vesca* L. (Figure 1) is a perennial herb and the most common and widely distributed species in the genus *Fragaria* (Rosaceae) (Hilmarsson et al., 2017). Besides Scandinavia, it occurs throughout most of the Holarctic including large parts of Europe, Asia and North America.

The woodland strawberry grows naturally in various habitats ranging from half sunny to sunny. It is frequent in open woodland and present in hedgerows, roadsides, pastures, edges of dense forests and farmland from the lowlands up to the subalpine zone where it prefers mountain slopes and thinner forest stands with favourable light conditions (Maliníková et al., 2013; Roiloa and Retuerto, 2007; Schulze et al., 2012).

This species is an erect rosette plant and a hemicryptophyte, in which overwintering buds are located at ground level and winter green leaves are produced which wither in the spring period (Darrow, 1966; Maliníková et al.,

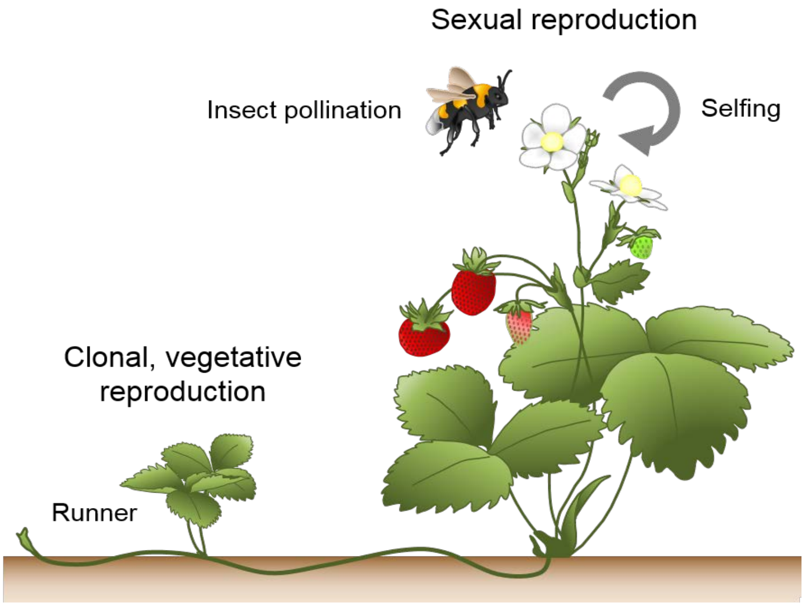


Figure 2. The woodland strawberry (*F. vesca*) primarily propagates by producing a high number of runners. For the sexual reproduction, the bisexually flowers are pollinized by insect pollinators and in rare occasions by selfing. After successful pollination the flowers yield fleshy accessory fruits containing the viable seeds. Illustration: Daniela Weber

2013). After winter, the axillary buds can develop either into rounded trifoliate leaves, stolons for runner production or an inflorescence (Darrow, 1966; Schulze et al., 2012). Across our study area (central Sweden), as well as much of this species' European distribution, flowers are produced throughout the entire growing season from April until September, with a clear peak in early June (Maliníková et al., 2013). The white bisexual and partly self-compatible flowers are generally pollinated by insects, although selfing also commonly occurs alongside cross-pollination (Figure 2). The fleshy accessory fruits are animal dispersed (Johnson et al., 2014), but the main propagation is vegetative which ensures survival in conditions unfavourable for sexual reproduction (Maliníková et al., 2013). Clonal reproduction takes place via runners which root and grow quickly into self-sustaining plants (Figure2). The woodland strawberry can produce a high amount of runners connected by above-ground stolons. Frequently in the wild, and especially in cultivation, this can lead to a fairly large clonal system with a thick carpet of interconnected runners scattered in space (Schulze et al., 2012). Eventually, these connections may disappear to leave independent plants.

In Europe, woodland strawberry has been grown in gardens at least since the Roman era (Liston et al., 2014; Wilhelm and Sagen, 1974). It has been valued for its small, soft, highly aromatic fruits with intense flavour, but has also been regarded as a medicinal herb species since the antiquity (Maliníková et al., 2013). Cultivated mainly in gardens of monasteries and castles, there was a shift to larger scale commercial horticulture of strawberries during the 16th century. Strawberries were sold at street markets as a snack or refreshment, making the strawberry no longer a product eaten by an elite (Vergauwen and De Smet, 2019; Wilhelm and Sagen, 1974).

The modern cultivated garden strawberry, *Fragaria* × *ananassa* (Duchesne) originated in the midst of the 18th century (Liston et al., 2014). In the early 18th century *Fragaria chiloensis* ((L.) Duchesne) was brought from Chile to France to impress the French King Louis XV. However, only female plants of this dioecious species made their way to Europe, so to gain cross-fertilization French breeders started to cultivate *F. chiloensis* together with *F. virginiana* (Duchesne), a North American strawberry species brought to Europe in the 16th century (Darrow, 1966; Liston et al., 2014; Vergauwen and De Smet, 2019). With the resulting hybrid, the garden strawberry was born and from then onward spread over the world replacing the woodland and other wild strawberries in markets (Vergauwen and De Smet, 2019).

The woodland strawberry has turned into a niche crop with limited cultivation in some European countries (Italy, France, Austria, and Poland). The fruits are very delicate, easily damaged, and mould quickly resulting in poor shelf life and shipping quality and, thus, are typically only available for local markets (Chambers et al., 2018; Doumett et al., 2011; Nin et al., 2018). However the fruit quality attributes such as the intense taste of the woodland strawberry are unique compared with those of the garden strawberry (Doumett et al., 2011; Ulrich et al., 2007) and provide an excellent source of bioactive compounds (D’Urso et al., 2018). The increasing awareness of health, nutrition and interest in “super foods” and local production has positioned the woodland strawberry as a fruit crop with a growing market demand, and as such with increasing economic potential. In demand by the food industry as well as the fresh market, woodland strawberry cultivation could provide an opportunity for strawberry growers to move into premium markets by producing gourmet, speciality strawberries (Chambers et al., 2018; Dias et al., 2016; Jurgiel-Małecka et al., 2017).

Besides its rediscovery as a valuable fruit crop, the woodland strawberry has a prominent position among the members of the Rosaceae family in plant science. It was selected as a genomic reference for the garden strawberry (*Fragaria* × *ananassa*) and functions as an emerging research model also for other species of the Rosaceae (Liston et al., 2014; Longhi et al., 2014; Shulaev et al., 2010). The Rosaceae family encompasses a range of economically important crop species such as apple (*Malus domestica*, Borkh.), peach (*Prunus persica*, (L.) Batsch), raspberries (*Rubus idaeus*, L.) and roses (Rosa spp.). The market is constantly increasing its demands in terms of quality and quantity, which has prompted the need to continually produce improved flowering and fruiting varieties of strawberry and rosaceous fruit crops generally (Hilmarsson et al., 2017; Longhi et al., 2014).

The woodland strawberry is a very attractive system for ecological and evolutionary studies due to its wide natural variation. A considerable ecological amplitude is found within this species since it adapted naturally to broad range of temperate habitats and elevations (Amil-Ruiz et al., 2011; Johnson et al., 2014; Liston et al., 2014). It provides the opportunity to investigate genetic variation for climatic tolerance (Egan et al., 2018; Rohloff et al., 2012), disease/pest resistance (Amil-Ruiz et al., 2011), and yield-associated traits (Chen et al., 2018; Kang et al., 2013; Mouhu et al., 2013). Further reasons why the woodland strawberry has repeatedly been proposed as an excellent research model, are:

- It is diploid with a small and sequenced genome (2n=14, 206Mb) (Longhi et al., 2014)
- It has a small, compact growth habit and is easily grown under controlled conditions
- It can be propagated both asexually (runners) and sexually (seeds) with a short seed-to-seed life cycle (Hilmarsson et al., 2017; Johnson et al., 2014; Schulze et al., 2012)

The genome sequence of the woodland strawberry brought development of a large number of genetic markers, efficient genetic transformation protocols and the availability of extensive transcriptome data (Hadonou et al., 2004; Oosumi et al., 2006; Sargent et al., 2004; Veilleux et al., 2012). Furthermore, the recent successful application of the (CRISPR)–Cas9 system (high efficient genome editing technology) to the woodland strawberry was recently reported (Zhou et al., 2018). All these tools allow the possibility to unravel physiological, metabolic and developmental processes, which have, for instance, already permitted greater insight into the molecular mechanisms underpinning flowering (Hollender et al., 2014; Mouhu et al., 2013), fruiting (Kang et al., 2013) and the circadian clock (Chen et al., 2018).

In some cases, the woodland strawberry has already been used as a source of genetic traits for introgression into strawberry cultivars (Chambers et al., 2018; Liston et al., 2014). New breeding strategies such as genetic marker-assisted breeding promise to significantly speed up this process, especially since different ploidy levels are involved (diploid woodland strawberry, octoploid garden strawberry) (Chambers et al., 2018; Longhi et al., 2014; Nakaya and Isobe, 2012;

Schaart et al., 2004). As a maternal ancestor of *F.* × *ananassa*, the woodland strawberry is highly congenic with the octoploid cultivated garden strawberry (Shulaev et al., 2010; Tennessen et al., 2014) and discovering strawberry orthologous genes will not only help molecular studies, but, can also be a useful tool to produce new strawberry varieties. Other methods such as genome editing or ectopic expression of heterologous genes, could indeed also help to produce important varieties of this crop. But transgenic modification in a fresh fruit is far from being accepted safe for human consumption (Amil-Ruiz et al., 2011; Nakaya and Isobe, 2012; Schaart et al., 2004).

3.2 Establishment of the Common Garden

For the experiments performed in this thesis involving wild plant genotypes, a collection of wild woodland strawberry genotypes was established in a common garden.

A total of 100 accessions of wild woodland strawberry were collected from random, geographically distinct locations (Fig. 2) across Uppsala County (Sweden) in early spring of 2012. Uppsala County encompasses an area of 8,209 square km and the distances between the individual sampling locations varied between 7 and 40 km. At each sampling site, a runner was collected from a randomly selected plant, representing one plant genotype, in order to maximize the geographic resolution of the sampled area. This geographic area is of particular climatic significance, since it represents a transition zone between southerly continental and northerly boreal environmental zones (Metzger et al., 2005). Accordingly, high levels of neutral genetic differentiation between populations in this region has indeed recently been established (Hilmarsson et al., 2017) as part of a wider microsatellite marker analysis of *F. vesca* genotypes.

The collected plant genotypes were propagated via runners for three vegetative generations and subsequently transplanted into the common garden in September 2012. The common garden (Figure 3) was organized in a randomized block design (one runner per plant genotype per block, 40 blocks),

Figure 3. Setup of the experimental plants in the common garden in Krusenberg, 15 km south of Uppsala, Sweden. Photo: Paul Egan

and located in an open agricultural field with sandy soil (N59.741°, E17.684°), 15 km south of Uppsala. The space was covered with fabric mulch (Weibulls Horto) to reduce weed densities and was manually weeded when necessary, but no irrigation or fertilizer was applied. The freshly planted strawberry runners were then allowed to establish and grow for two seasons before they were used in the experiments. At the beginning of the experiment 86 of the planted genotypes had still a full replication (n=10), since some of the plants died during the establishment.

3.3 The Insect System: the Beauty(ies) and the Beast(s)

The leaf beetles *Galerucella tenella* L. (Coleoptera: Chrysomelidae) and *Galerucella sagittariae* Gyllenhaal belong to the family Chrysomelidae, one of the very species-rich families of herbivorous beetles. *Galerucella* beetles occur mainly in moist habitats like marshy riversides and use at least seven distantly related plant families as food plant (Borghuis et al., 2009; Hambäck et al., 2013; Silverberg, 1974). Many of the plant species serving as food plants co-occur in the same habitat and several can be present within close distance, which means that different species of *Galerucella* beetle can still exhibit broad habitat overlap as well. *Galerucella sagittariae* as well as *G. tenella* are the more oligophagous species of the genus *Galerucella* (Borghuis et al., 2009; Hambäck et al., 2013; Nokkala and Nokkala, 1998).

Commonly found on marsh cinquefoil (*Potentilla palustris*, L. Scop.) and tufted loosestrife (*Lysimachia thyrsiflora*, L.), *G. sagittariae* feeds on various Rosaceae, Primulaceae and Polygonaceae species (Nokkala and Nokkala 1998; Verschut and Hambäck 2018). *Galerucella tenella*, also known as the strawberry leaf beetle, is found on different species of the Rosaceae family and forms abundant populations mainly on meadowsweet (*Filipendula ulmaria*, L.), but also on cloudberry (*Rubus charnaemorus*, L.), arctic bramble (*Rubus arcticus*, L.), raspbererry (*Rubus idaeus*, L.) marsh cinquefoile (*Potentilla palustris*, L.) common lady's mantle (*Alchemilla vulgaris*, L.) as well as wild *Fragaria* spp and the cultivated garden strawberry *(F. x ananassa*, Duchesne) (Hambäck et al., 2013; Nokkala and Nokkala, 1987; Stenberg et al., 2006). Wild meadowsweet serves as the main food source, on which population densities of the strawberry leaf beetle fluctuate asynchronously in space and time and sudden increases occur frequently (Hambäck et al., 2006; Stenberg et al., 2006).

Both leaf beetle species can significantly reduce flower and leaf area through feeding and as such plant fitness (Figure 4). It can reach pest status especially in organic strawberry cultivations in Scandinavia, Finland (Stenberg, 2012, 2014), the Baltic States (Kaufmane and Libek, 2000; Petrova et al., 2006), and Russia (Bulukhto and Tsipirig, 2004).

Figure 4. Feeding damage caused by larvae and adult beetles of the strawberry leaf beetle (*G. tenella*) on woodland strawberry (*F. vesca*,) A) leaves and B) flowers. Photos: Daniela Weber

The life cycles of the two *Galerucella* beetles (Figure 5) are very similar and both are univoltine species that hibernate as adults in the topsoil and usually re-emerge during April to May when the plants start to regrow after winter. A few days after they emerge from hibernation, the adult beetles mate and the eggs are laid directly on the leaves and petioles of the plant they feed on (Stenberg et al., 2006; Verschut and Hambäck, 2018). The larvae hatch after approximately two weeks depending on the climatic conditions (Stenberg et al., 2006). Like the adult beetles, the larvae feed on the leaves and flowers and occasionally gnaw the fruit surface causing brown scaring or even tunnel into ripe strawberries (Parikka and Tuovinen, 2014). After 2-4 weeks of feeding the larvae leave the host plant to pupate in the upper soil layer from early July until mid-August. 2–3 weeks after pupation, the next generation emerges (Fors et al., 2016; Stenberg et al., 2006; Verschut and Hambäck, 2018).

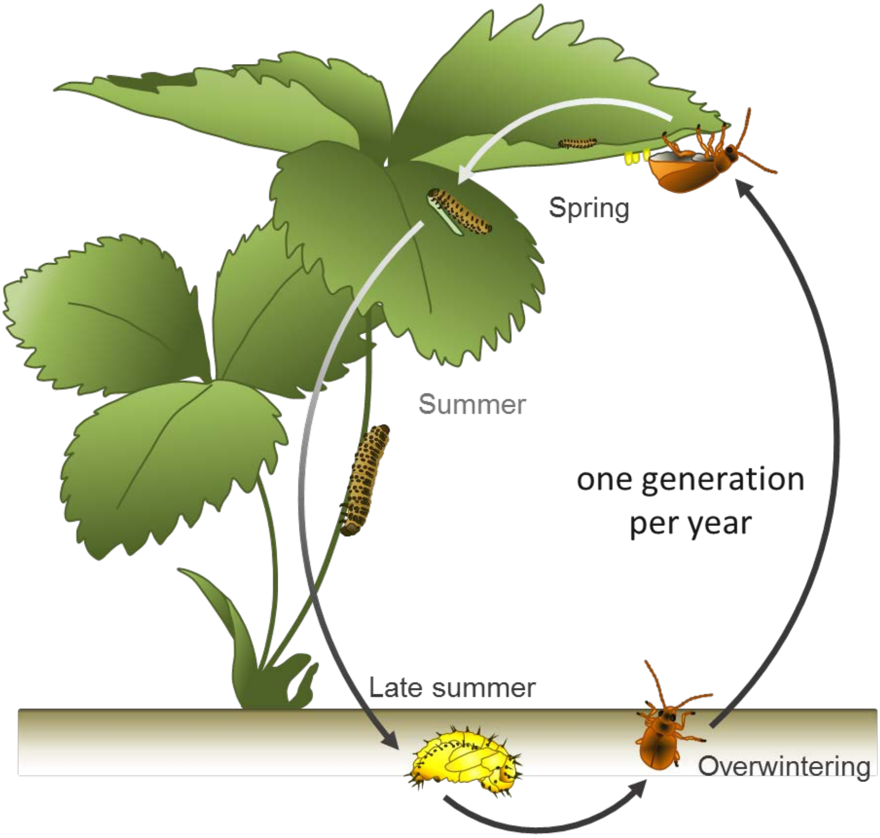


Figure 5. Seasonal life cycle of the strawberry leaf beetle (*G. tenella*) feeding on a woodland strawberry (*F. vesca*). Egg laying takes place in spring and is followed by larval development in summer. The beetle larvae pupate in the soil and the new generation of adult beetles overwinter in the soil after a short feeding period in autumn. Illustration: Daniela Weber

One of the most important regulatory factors of the strawberry leaf beetle (*G. tenella*) in wild populations as well as in strawberry plantations is the parasitoid *Asecodes parviclava* Thompson (Hymenoptera: Eulophidae) (Stenberg, 2012, 2014). It is so far the only known larval endoparasitoid of the strawberry leaf beetle. The only other known species attacked by *A. parviclava* are two other closely related chrysomelids; *Galerucella calmariensis* (L.) and *Galerucella pusilla* (Duftschmid). (Hambäck et al., 2013; Hansson and Hambäck, 2013). Their habitat can overlap with the strawberry leaf beetle, but both of the later species are monophagous on a different wetland plant, the Purple loosestrife (*Lythrum salicaria*, L.). The parasitoid appears to be present in most areas with *G. tenella*, but there seems to be a latitudinal shift in parasitism rates throughout Sweden with typically higher rates in northern and lower rates in southern localities (Fors et al., 2016; Hambäck et al., 2006).

Asecodes parviclava is a small (<2mm) gregarious, konobiont endoparasitoid. The female parasitoid attacks the early larval stage of its beetle host by inserting one or more eggs into the larva's body. When the eggs hatch the neonate parasitoid larvae start to feed on the haemolymph of the host larva. Appearing to develop normally, the parasitized larva continues to feed and grow until the last stages of parasitism. The parasitoid larvae eventually consume all the tissue of their host larva from within, at last killing the host larva. The remaining cuticle of the host larvae turns into a hollow, mummified black shell around the pupating neonate parasitoids, forming the so called mummy. Most of the parasitoids overwinter as pupae inside the mummified host larva and emerge as adults during the next summer, when new beetle larvae are available Emerging females may parasitize new host larvae immediately, with no foraging needed (Fors et al., 2016; Hambäck et al., 2013; Hansson and Hambäck, 2013; Stenberg and Hambäck, 2010).

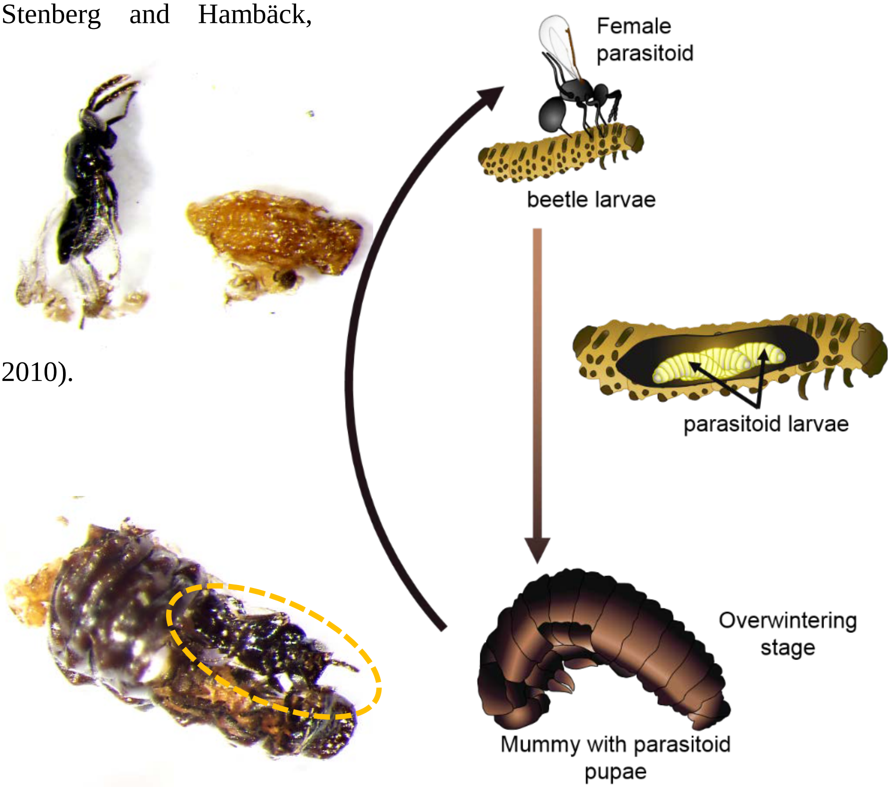


Figure 6. Life cycle of *Asecodes parviclava*. The female parasitoid inserts the eggs into the body of an early stage beetle larvae. The parasitoid larvae develop inside the growing host, which continues to grow and feed. The host turns into a hollow mummy in which the parasitoid larvae pupate and from which the adults subsequently emerge. On the left, picture of adult parasitoid and pupa (above) and mummified host showing an adult parasitoid (bottom). Illustration and photos: Daniela Weber

3.4 Insect Collection

For the experiments performed in this thesis, required insects were collected from different localities in Sweden in preparation of the experiments each season. The insect herbivores and the parasitoid used in this study are native to Scandinavia and are sympatric in large areas of their distribution.

The Galerucella Leaf Beetles

The two herbivorous beetle species were collected from natural populations in the vicinity of Uppsala from their respective main food plant in the developmental stage that served the purpose of the experiment best:

- *Galerucella sagittariae* was collected as early instar larvae from Marsh cinquefoil (*Comarum palustre*) and tufted loosestrife (*Lysimachia thyrsiflora*) and used directly in the experiment (pollination test) and obtained as eggs on Marsh cinquefoil (*Comarum palustre*) cuttings that were collected from natural Marsh cinquefoil stands and reared indoors until the larvae hatched.
- Strawberry leaf beetle (*G. tenella*) was collected as mating couples. Selecting already mating beetles ensured that the collection contained egg laying females. The gravid female beetles were either separated from their partner and used directly (oviposition experiment), or placed with all collected beetles in meshed cages in a greenhouse (15°C, LD 16:8 h photoperiod, 80% RH) to allow them to lay eggs on a random mix of potted woodland strawberry. The plants were renewed after 24h and the eggs on the strawberry plants were kept in the greenhouse until the larvae hatched.

The Parasitoid

The parasitoid *Asecodes parviclava* was reared from late instar larvae of the strawberry leaf beetle. The beetle larvae were collected from natural beetle populations on meadowsweet growing in the area of the Skeppsvik Archipelago, Sweden and kept on meadowsweet cuttings at room temperature until pupation. The mummies with the parasitoid pupae were stored during the winter at a shaded and sheltered location outdoors until spring, when the adult parasitoids were expected to emerge. Upon emergence, the adult parasitoids were fed with diluted honey and placed together to allow mating. The female parasitoid (distinguishable by their point abdomen and bigger size) were used 3-4 days after they emerged for the parasitism experiment.

4 Flowers and Bees: Effect of Herbivory on Pollination

Maintaining effective insect pollination services is critical for all pollinator-dependant crops such as strawberry (Kleijn et al., 2015; Ollerton, J., Winfree, R., and Tarrant, 2011). However, pollinator abundance and diversity is thought to be in decline worldwide, and the application of chemical pesticides is seen as one important factor advancing this decline. Pesticide-free farming can in theory favour higher pollination abundance, but in turn also higher herbivore densities (Stanley et al., 2015). Herbivory, especially of the flowers, can corrupt the plants' attraction to pollinators and keep pollinators from visiting herbivore-damaged crops (Lucas-Barbosa et al., 2015; Tsuji et al., 2016). In this study direct and indirect effects of floral herbivory on natural pollination and fruit quality of the woodland strawberry (*Fragaria vesca*) was tested. Furthermore the natural herbivore resistance of wild woodland strawberry genotypes was investigated in order to survey the potential for re-introducing resistance in commercial strawberry varieties as alternative pest control to chemical pesticides.

4.1 Methods

Pollinator Response to Manually Damaged Flowers

The pollinator response to the condition of the flowers (intact or damaged) was tested by observing the number of pollinator visits to treated or untreated flowers of the experimental plants. Potted woodland strawberries of the commercial variety 'Rügen' (n=32, Mälarö Odling AB, Ekerö, Sweden) were placed outside a caged area in order to allow visits of wild pollinating insects and to simultaneously exclude birds that could consume the ripened fruits. For each plant individual, half of the open flowers were left undamaged and half were manually damaged by perforating the centre of one petal with a pencil. Observation of flower-pollinators interactions was conducted immediately after the preparation of the flowers in the early morning and lasted as long as the pollinators were flying.

Effects of Herbivory on Pollination Success and Fruit Development

The effects of herbivory on pollination success and fruit development was tested by treating the flowers of 32 plant individuals of the woodland strawberry ‘Rügen’ in three different ways: (1) In the control treatment the flowers were left undamaged and pollinated openly. In the other two treatment the flowers were exposed to an early instar *Galerucella sagittariae* larva (see 3.4. Insect collection) for 24h and (2) either pollinated openly or (3) pollinated by hand with pollen taken from both the flower’s own anthers and from three other ‘Rügen’ individuals. From the 32 plant individuals each plant individual received all 3 treatments and from the 3 different treatments each treatment type was applied to 2 random freshly opened flowers. During the whole experiment, the treated plants were placed in a caged outside area. Successful pollinated flower pistils turn darker and pollination success was measured by estimating the percent of darkened and thus successfully pollinated pistils. Fruit development was measured by weighing the ripe fruits and counting the number of deformations per fruit.

Resistance of Wild Woodland Strawberry

The herbivore resistance among woodland strawberry was tested with *G. sagittariae* (see 3.4. Insect collection) as well as with the strawberry leaf beetle *G. tenella* (see 3.4. Insect collection). In both cases leaf tissue was used to test for herbivore resistance due to the feeding habit of the leaf beetle larvae. Hatching before the onset of flowering, the mobile larvae utilize the rather small strawberry flowers in addition to the leaves in their later stage of their development.

For the experiment with *G. sagittariae,* runners from 20 woodland strawberry genotypes in the common garden were used to produce ten genetically identical replicates of each genotype. Each of the plant individuals received 4 early instar *G. sagittariae* larvae, and was bagged and kept outside in the caged area during the experiment. After 3 weeks the larvae were removed and the proportion of damaged leaves was used as measure of plant resistance to *G. sagittariae*.

In the case of the strawberry leaf beetle, newly hatched larvae were reared individually on leaf material from the assigned woodland strawberry genotype. Besides ‘Rügen’, the detached leafs were obtained from 19 wild genotypes in the common garden. The leaves were exchanged every third day and the larvae were kept in a climate chamber (15°C, LD 16:8 h photoperiod, 80% RH) until pupation. The date of pupation was noted for each larvae and the larval development time (days to pupation) was used as a measure of plant resistance to *G. tenella*.

4.2 Results and Discussion

Pollinator Preference for Undamaged Flowers

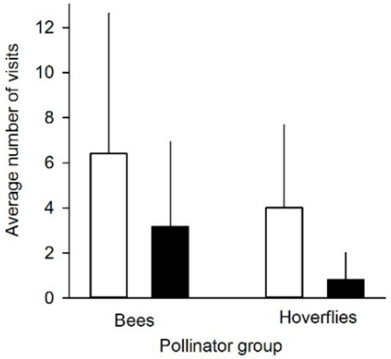


Figure 7. Average number of pollinator visits to undamaged flowers (white bars) and manually damaged flowers (black bars). Pollinators were divided into two groups: bees (n = 5) and hoverflies (n = 17). Mean + SD.

Intact control flowers were visited more frequently than damaged flowers, mainly due to hoverfly (Syrphidae) avoidance rather than by bees (superfamily Apoidea). Native hoverflies were the dominating pollinator group in this observation and are known to play an important part in pollinating wild strawberry populations as well as organic outdoor plantations (Albano et al., 2009; Sharma et al., 2014). A clear decrease in the pollination success was observed for open pollinated flowers damaged by *G. sagittariae* larvae, thus the reduced visitation by hoverflies due to herbivore damaged flowers could have important impacts on the pollination services in strawberry cultivation. Looking also at olfactory clues besides the here studied visual clues might bring additional insight in the mechanism underlying pollinator choice (Gerber and Smith, 1998; Klatt et al., 2013; Primante and Dötterl, 2010; Sutherland et al., 1999), since both these cues are known to be altered by herbivory (Hoffmeister et al., 2016)

Pollination Success and Fruit Yield

Intact, open pollinated flowers yielded heavier fruits than the other treatments involving flower damage by herbivory and had a higher pollination success then the open pollinated, herbivore damaged flowers. In general, low pollination success of strawberries was associated here with smaller and more deformed fruits. Both fruit size and shape are characteristics of high economic importance for growers. Herbivory to the flowers not only decreased fruit quality directly but also indirectly by reducing pollination success and thus could potentially have major economic consequences. A previous study, which took reduced fruit size and shape into account, showed that insect pollinators contributed 39% to a total of 2.90 billion US$ made from selling 1.5 million tons of strawberries in the EU in 2009 (Klatt et al., 2013).

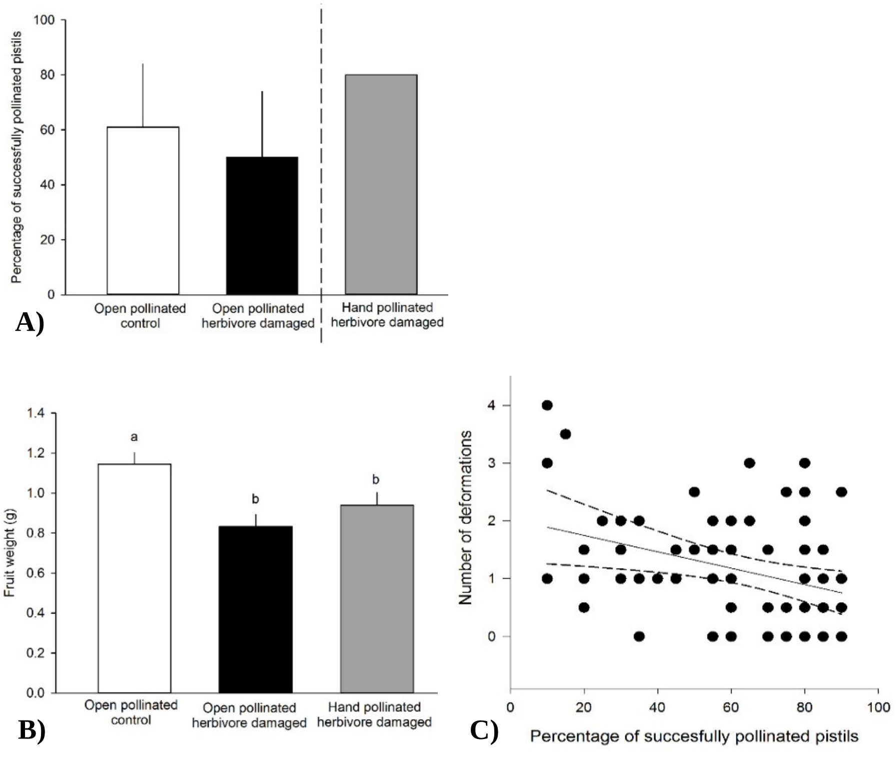


Figure 8. **A)** Percentage of pollinated pistils for either undamaged, open pollinated flowers (white bars) or *G. sagittariae* damaged, open pollinated flowers (black bars). *G. sagittariae* damaged hand pollinated flowers were treated until they reached 80% pollinated pistils per flower (gray bar), thus were excluded from analysis. Mean + SD. **B)** The effect of treatment on the fruit weight (g) of *F. vesca*. Different letters indicate statistically significant differences in fruit weight among the t treatments ($P < 0.05$). LS means **C)** deformation count of *F. vesca* fruits produced from flowers with varying degrees of pollination success.

In both tested cases of *G. sagittariae* and the strawberry leaf beetle, the herbivore resistance varied among the 20 tested woodland strawberry types. The proportion of leaves damaged by *G. sagittariae* as well as the developmental time of *G. tenella* varied significantly between the genotypes, indicating genetic variation in plant resistance.

Breeding for increased herbivore resistance is an increasingly important method to mitigate herbivory-mediated pollination deficiency and may be an alternative to chemical pesticides in pollinator dependent crops such as strawberries. Pesticides, though an effective way to control herbivores, have in most cases unwanted, harmful side effects on pollinators (Stanley et al., 2015).

The results of this study indicate that even limited herbivore damage has the potential to reduce pollination services and in turn quantity and quality of the yield. Thus, the existence of genetic diversity in herbivore resistance constitutes an important finding since it opens up the possibility for reverse breeding to restore high resistance in modern strawberry cultivars (Andersen et al., 2015; Palmgren et al., 2015). An important question for future research is whether plant traits deterring herbivores might cause trade-offs for pollinators (Adler, 2000; Adler et al., 2012).

5 Which Strawberry (Leaf) is Tastier? Plant Resistance against Strawberry Leaf Beetle

One way to decrease the dependency of cultivated strawberry on chemical pesticides would be to strengthen pest resistance in strawberry cultivars. Yet genetic resources within domesticated varieties are limited (Chen et al., 2015a). Wild crop relatives such as the woodland strawberry, which have remained untouched from domestication, are thus suspected to harbour high genetic variation in useful resistance traits.

Knowledge about insect herbivore resistance among wild woodland strawberries is scarce and further exploration of the wild germplasm is needed. Over eighty wild woodland strawberry genotypes from central Sweden were hence screened for intraspecific genetic variation in intrinsic herbivore resistance against the strawberry leaf beetle (*G. tenella*); a strawberry pest in Sweden and other parts of Northern Europe.

5.1 Methods

Plant Resistance among Woodland Strawberry

The herbivore resistance of wild woodland strawberry was screened among all the strawberry genotypes growing in the common garden with a full set of replication (n=10). The 86 strawberry genotypes were tested for their effects on strawberry leaf beetle larvae in terms of larval survival, developmental time and pupal weight. The freshly hatched larvae (see 3.4. Insect collection) were kept individually in a climate chamber until the adult stage (15°C, LD 16:8 h photoperiod, 80% RH) and provided with fresh leaf material from the assigned strawberry genotype. Upon pupation, the date and weight of each pupae was noted as well as the corresponding sex of the adult beetle. The developmental time (hatching to pupation) was divided by pupal weight and the inverse of the resulting growth rate was used as proxy for the herbivore resistance level for each plant genotype. Low larval growth rate corresponds to high level of resistance and vice versa.

Oviposition Preference and Hatching Success

Gravid *G. tenella* females (see 3.4. Insect collection) were tested for whether they discriminated for their egg laying between strawberry genotypes identified as susceptible and resistant. Based on the larval growth rate from the resistance screening, a subset of 16 plant genotypes was selected, including 8 susceptible (low level of resistance) and 8 resistant (high level of resistance) against the strawberry leaf beetle. Runners were obtained from the common garden to gain 10 genetically identical, equal sized replicates of each selected genotype. One resistant and one susceptible strawberry genotype were randomly paired and offered to a single, gravid strawberry leaf beetle to oviposit freely. The cages with plants and beetle were placed outside for the duration of the experiment. After 48h, the females were removed and for each plant, the number and the position (leaf blades or leaf petioles) of the eggs was noted. The plants were placed back into their cages outside and the hatching success of the eggs was measured by allowing the eggs to hatch naturally and counting the first round of neonate larvae (<24 h) for each plant individual. Plants and G. *tenella* females were used only once in each oviposition choice trial.

5.2 Results and Discussion

Plant Resistance among Woodland Strawberry

The strawberry leaf beetle growth rate varied markedly between the tested wild woodland strawberry genotypes. Strawberry genotypes that supported higher herbivore growth rate also contributed to higher survival of the strawberry leaf beetle. In addition, when the two factors of the growth rate, larval development time and pupal weight, are considered separately, each of them shows significant genetic variation. The mortality during the experiment was generally low, however, the significantly reduced herbivore growth rate on certain genotypes might further increase mortality in a more natural setting due to prolonging the window of vulnerability of *G. tenella* to natural enemy attack (Clancy and Price, 1987; Williams, 1999).

Ranging from susceptible to highly resistant, the large gradient in herbivore resistance found in this screening of wild woodland strawberry suggests that an untapped genetic resource in herbivore resistance is available in wild woodland strawberry germplasm. The Nordic wild woodland strawberry populations sampled for this study represent only a small fraction of this widely holoarctic distributed plant species and it suggests that even higher variation could be obtained if germplasm from the whole distribution was screened.

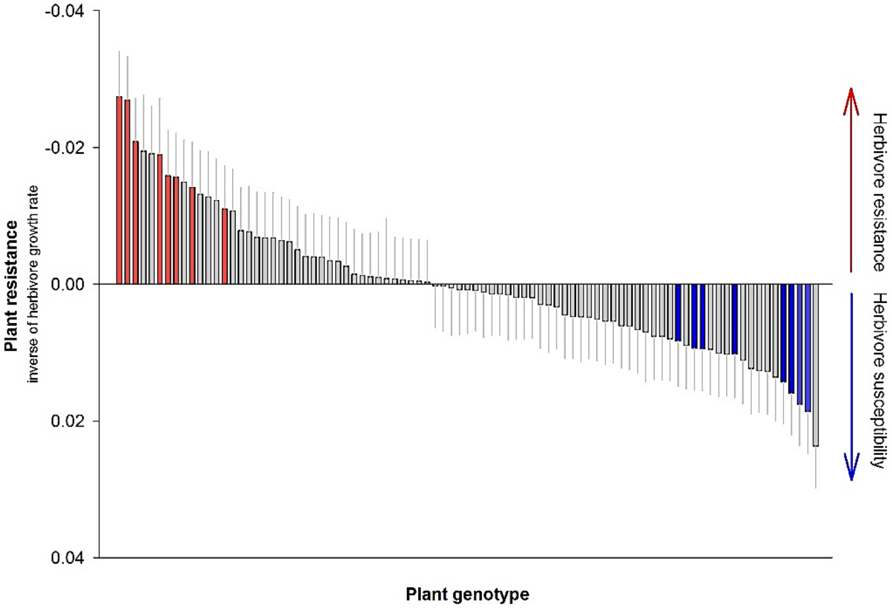


Figure 9. Genetic variation in plant resistance (antibiosis) against *G. tenella* in 86 wild *F. vesca* genotypes. Antibiosis was measured as inverse of herbivore performance. Larval growth rate (pupal weight divided by the larval development time from hatching until pupation) was used as a measure of herbivore performance. Mean larval growth rate on each plant genotype is compared to the overall mean (standardized on zero) of all 86 plant genotypes. Larval growth rate lower than the overall mean (negative value) indicate more resistant plant genotypes, while positive values indicate more susceptible plant genotypes. The red and blue bars are the plant genotypes selected for the oviposition preference testing and egg survival. Predicted mean (total genetic value) + SE.

Oviposition Preference and Hatching Success

The oviposition preference of the strawberry leaf beetle varied between susceptible and resistant wild woodland strawberry genotypes. The female beetles showed a clear preference for susceptible strawberry genotypes with a low level of resistance, and thus an ability to make optimal choices for their offspring (cf. the mother knows best hypothesis) (Jaenike, 1978; Valladares and Lawton, 1991). Egg hatching success was unaffected by plant resistance level, indicating that plant defences in this herbivore-plant system are more directed against the larval stage than the egg stage of the beetle. The fact that oviposition preference and resistance level correlate will be beneficial from a future breeding perspective, as this opens up the possibility to simultaneously optimize both aspects of resistance, and in this way provide robust resistance against the target herbivore (Stenberg and Muola, 2017). Given the continuous need to develop resistant strawberry cultivars, the inclusion of resistant wild woodland strawberry germplasm into strawberry breeding programs is one promising way to breed modern cultivars of the garden strawberry (*Fragaria x ananassa*) with improved resistance and reduced need for chemical pesticides.

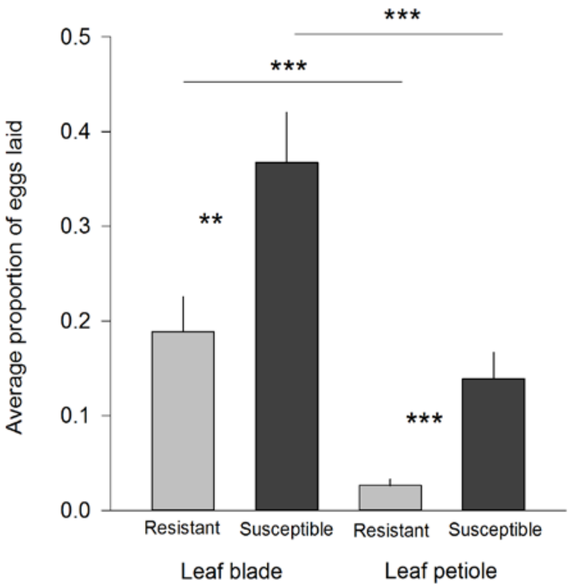


Figure 10. Effect of plant resistance on the interactive effects of leaf tissue (leaf blade vs. leaf petiole) and plant antibiosis level on oviposition preference of *G. tenella*. Pairwise a priori contrast comparisons were conducted to compare differences between resistant leaf blade vs. resistant leaf petiole, susceptible leaf blade vs. susceptible leaf petiole, resistant leaf blade vs. susceptible leaf blade and resistant leaf petiole vs. susceptible leaf petiole. Estimated marginal means + SE. ** 0.05 < P < 0.001; *** P <0.001.

6 You are What You Eat: Tritrophic Role of Plant Resistance

Within plant tissues a wide variety and function of chemical compounds can be found encompassing primary metabolites involved directly in growth and metabolism and secondary metabolites involved in plant defence against biotic or abiotic stresses. The quality of plant material, in terms of direct herbivore resistance and its general chemical composition, can play an important role in shaping interactions between herbivores and their natural enemies such as parasitoids. In particular, it is increasingly acknowledged that plant herbivore defence compounds may also impair the performance of parasitoids that attack the insect herbivores.

Minimizing conflicts between breeding for higher plant resistance and biological control is crucial to help meet the demand for sustainable and more environmentally-friendly crop protection (Chen et al., 2015a; Peterson et al., 2016). Therefore it is important to evaluate plant herbivore resistance from a biocontrol perspective. In this study the parasitoid fitness of the larval endoparasitoid *Asecodes parviclava* was monitored for sixteen wild woodland strawberry (*Fragaria vesca*) genotypes showing a large gradient in resistance against the parasitoid's host, the strawberry leaf beetle (*Galerucella tenella*). Additionally, the survival of the parasitoid was related to a broad metabolomic profile of primary and secondary plant compounds of the strawberry genotypes in order to identify potential links between the plant compounds and survival rate. Although primarily studied separately to date (van Geem et al., 2016), this study sought to examine the combination of primary and secondary plant compounds in order to gain a more holistic understanding of the impact that plant quality has on the development of parasitoids,

6.1 Methods

Experimental Setup for the Parasitism Experiment

For testing the effects of plant quality on parasitoid fitness, early instar larva of the strawberry leaf beetle were one by one parasitized, each by a single female *A. parviclava* (see 3.4. Insect collection). The parasitized larvae were reared individually on leaf material from the assigned genotype. The same subset of 16 strawberry genotypes (8 resistant, 8 susceptible genotypes) was used as in the oviposition preference experiment (see 5.1). The larvae were place in a climate chamber (15°C, LD 16:8 h photoperiod, 80% RH), checked on daily and the leaves were exchanged for fresh material every third day. Upon pupation, it was noted whether the host larva died before pupation, the parasitism was successful (i.e. the host larva developed into a mummy) or whether the beetle was able to overcome the parasitism (i.e. developed into a beetle pupa). The date of reaching the pupal stage was recorded to determine the developmental time from parasitisation to pupation. Each mummy was weighed and carefully opened after the winter to count the enclosed parasitoid pupae.

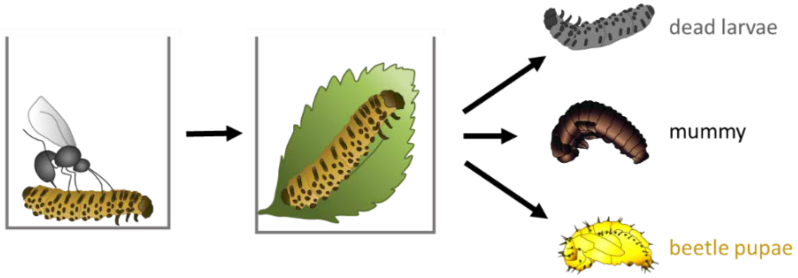


Figure 11. Conceptual illustration of the experimental setup. The beetle larvae (*G. tenella*) were parasitized individually and fed with fresh leaf material form the assigned woodland strawberry (*F. vesca*) genotype. The parasitized beetle larvae developed either into a beetle pupa, parasitoid mummy or died before pupation. Illustration: Daniela Weber

Metabolomic Analysis of the Tritrophic Effects of Food Plant Quality

The outcome of the parasitism, whether the parasitism was successful or not, clearly depended on the plant genotype the larva was feeding on. In order to know more about the underlying mechanisms, the three categories of the parasitism outcome (dead pre-pupal host larva, mummy, or beetle pupae) were statistically related to the metabolites present in the leaf material of the 16 strawberry genotypes. In order to do so, plant leaves were sampled from the same sixteen genotypes in the common garden which were exposed to strawberry leaf beetle feeding. The leaves were dried, grinded and sent to the

Swedish Metabolomics Centre, Umeå, Sweden for metabolomic profiling by GC/TOF-MS (gas chromatography/time of-flight-mass spectrometry). All files retrieved from the GC/TOF-MS were compared with libraries of retention time indices and mass spectra to identify as many compounds as possible. Partial least squares generalized linear models (PLS-GLM) successfully identified 39 compounds positively associated with at least one of three outcomes of the parasitism (host larvae died; larvae became a beetle pupa; larvae became a mummy).

6.2 Results and Discussion

Tritrophic Effects of Food Plant Quality

The different plant quality of the strawberry genotypes had a significant effect on the success and outcome of the parasitism. Interestingly the effect was not influenced by the direct plant resistance to the hosting leaf beetle, but rather the overall effect of plant quality and leaf content (primary and secondary plant metabolites) of the different plant genotypes determined the suitability of the herbivore as host for the immature parasitoid.

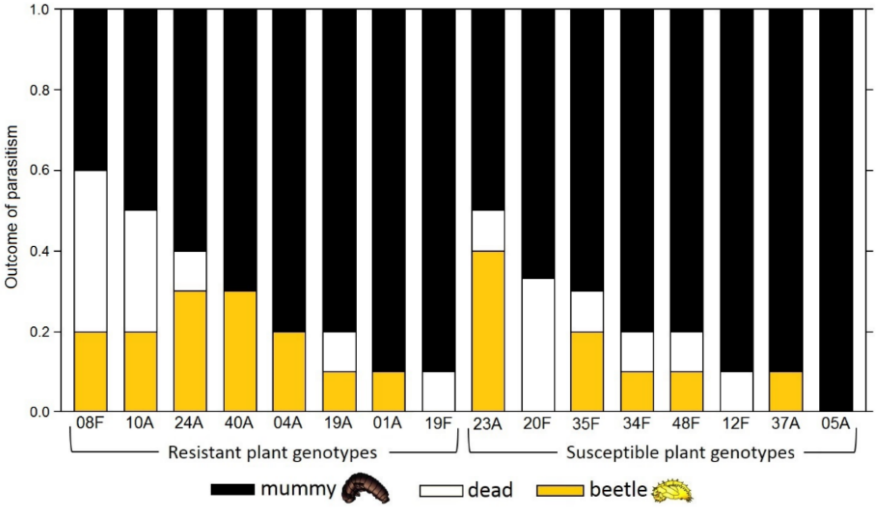


Figure 12. Outcome of parasitism in relation to plant genotype. Proportion of parasitized beetle larvae that became a parasitoid mummy (black), a beetle pupa (yellow) or died pre-pupa (white) across 16 wild *F. vesca* genotypes. Although there was no interaction between the outcome of the parasitism and plant herbivore resistance level, the data shown discriminated for the individual wild strawberry genotypes, (p < 0.05, multinomial logistic regression).

Although plant quality is an important factor that enables or hinders immature parasitoids to reach the pupal stage, other life history traits (developmental time, mummy weight, amount of pupae) were not affected. This indicates that they are more conserved life history traits in the parasitoid and certain physical thresholds must be met for completing the larval stage.

Non-targeted Metabolomic Profiling

Based on the GC/TOF-MS profiling, eighty-one compounds were overall detected in the tested plant material, encompassing a broad range of primary and secondary plant compounds

A subset of forty compounds were significantly associated with herbivore and/or parasitoid mortality. No overlap in compound association occurred between successful parasitism (i.e. parasitoid survival) and averted parasitism (i.e. beetle survival) as well as between all three outcomes (host larvae died; larvae became a beetle pupa; larvae became a mummy). Among the detected compound groups of primary metabolites (amino acids, carbohydrates, lipids, organic acids), carbohydrates were seemingly the most beneficial group for the successful parasitoid development. Besides representing a direct energy source, carbohydrates can also be converted into lipids. During development most parasitoid species rely on host lipids instead of synthesizing the lipids de novo and often manipulate the host's metabolism to ensure a sufficient lipid supply until pupation (Visser and Ellers, 2008).

Besides primary compounds, the leaf material also contained several detected phenolics and terpenoids, which are secondary metabolites often linked with plant resistance against herbivores. Interestingly the detected phenolic compounds were mainly positively linked with parasitoid survival, potentially making the host more susceptible for parasitism. The detected terpenoids on the other hand seemed to impair the development of the parasitoid *A. parviclava*.

Both primary and secondary plant compounds turned out to be equally important for the larval development of the parasitoid *A. parviclava*. It shows, that a wider range of plant compounds, instead of a small set of either primary or secondary compounds, need to be taken into account when studying the impact that plant herbivore resistance has on the development of parasitoids.

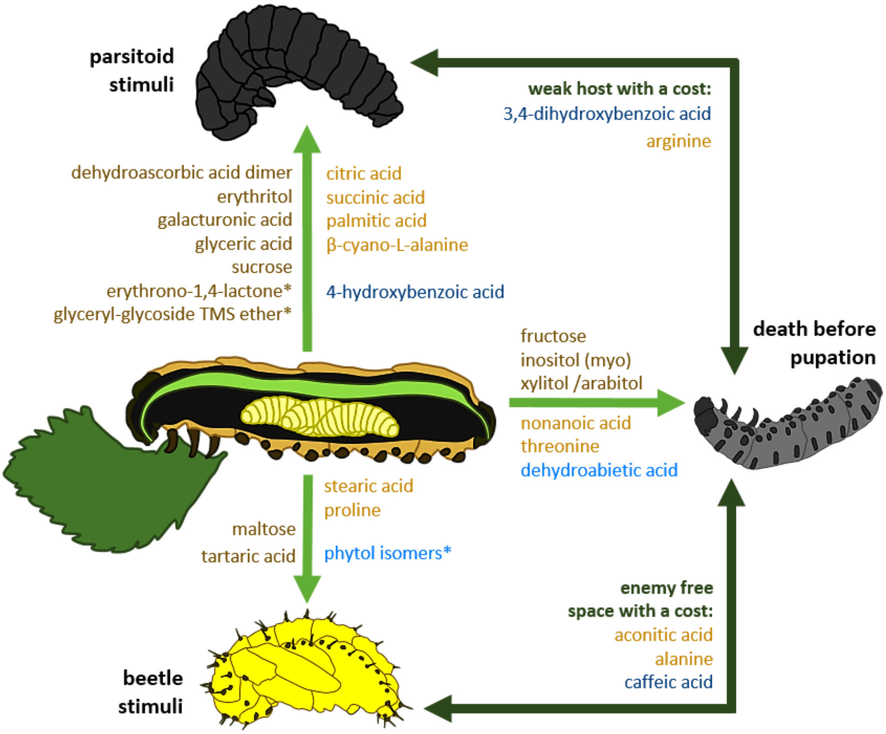


Figure 13. Schematic of the key plant compounds involved in the outcomes of the parasitism. Light green arrows indicate plant compounds promoting one outcome and dark green arrows promoting two outcomes. The plant compounds consist of primary compounds including carbohydrates (dark brown) and amino acids, lipids, organic acids (light brown) as well as secondary compounds including phenolics (dark blue) and terpenoids (light blue). Illustration: Daniela Weber

7 Conclusions and Future Perspectives

The results presented in this thesis demonstrate that the use of plant herbivore resistance as an IPM strategy has the potential to support the performance of natural enemies and maybe also pollination services.

Damage to the flowers of the woodland strawberry (*F. vesca*) caused by the leaf beetle larvae (*Galerucella* spp.) led to a change in pollinator behavior as well as pollination success. Flowers exposed to herbivory had less visitation by pollinators and the low pollination yielded smaller, deformed fruits. Even when pollination was ensured by hand pollination, the flowers damaged by the herbivore still generated smaller fruits. This finding indicates that herbivore damage not only imposed indirect effects via pollination, but also had direct effects on yield. So even if herbivore damage is incurred by flowers, this has the potential to impede the quantity and quality of yield with tentative economic consequences for the growers. Here only visual flower attributes were taken into account, including also olfactory clues might bring additional insight in repelling effect of herbivore caused floral damage.

This findings show that there is a need to control herbivore damage during flowering and, mindful of the need for pollinator conservation, to implement effective alternatives to insecticide use. Enhanced herbivore resistance in the crop itself presents a promising solution to jointly control pest insects and support pollination services, and thus ensure crop productivity. However, an important question that needs to be addressed in future research is whether changes in the crop’s defence traits interfere with pollination success, such as by effects on pollinator behaviour and/or fitness.

Restoring pest resistance in crop cultivars is a corner stone of IPM and wild crop relatives like wild woodland strawberries are predicted to show high heritable variation in resistance traits useful for crop improvement. In fact, the screening of wild accessions of woodland strawberry from central Sweden showed high variation in affecting the performance of the strawberry leaf beetle (*G. tenella*), a pest insect also occurring in cultivated strawberry, revealing

heritable genetic variation in herbivore resistance. Moreover the leaf beetles preferred to lay their eggs on plant genotypes that turned out to be susceptible to the strawberry leaf beetle. This synergy between performance and preference of the herbivore might furthermore help to decelerate adaptation of the pest to plant resistance, and thus to facilitate a more robust basis for pest control. Given that woodland strawberry is widely spread throughout most northern latitudes, even higher variation might be obtained were sampling to be extended to the whole distributional area of this plant species.

As a whole, the genetic variation identified in this study serves to strengthen the position of woodland strawberry as a key crop wild relative and its potential to contribute crop improvement programs for the domesticated strawberry. While genetic resources for pest resistance are limited within the domesticated strawberry (*Fragaria* × *ananassa*), this study suggests that wild woodland strawberry contains unexploited resources for resistance traits that are needed to reduce pesticide-dependence in strawberry cultivation. This finding can serve as cornerstone for wide fields of further explorations of herbivore-plant interactions in strawberry that can lead, for example, in the direction of “rewilding” the domesticated strawberry. An interesting aspect would be to screen the same set of accessions for their resistance against a pest from a different feeding guild such as a sucking insect to identify overlaps and potential interactions. To also put herbivore resistance into a spatial context, examining geographic patterns in wild plant populations could give insight into plant adaptation and evolution. The molecular side of this uncovered variation in plant resistance would be another aspect that could be addressed in future research to reveal the metabolomic and genetic mechanisms underpinning herbivore resistance in woodland strawberry.

In combination with biological control, a widely used key approach in IPM, plant resistance in a crop could be counterproductive and actually suppress sufficient natural enemy population build-up. In the woodland strawberry system, parasitoid performance was affected by plant-mediated variation in herbivore quality, which was evident by a significant link between parasitoid survival and foliar chemistry. In particular, of the examined compounds, carbohydrate levels appeared to be the most important for parasitism success. The metabolomics approach here taken indicated that consideration of a wider range of plant compounds, instead of a small sub-set of either primary or secondary compounds, proved beneficial towards identifying potential plant-mediated effects on parasitoid development. However, within the tested woodland strawberry collection, several genotypes with high herbivore resistance also yielded an elevated parasitoid performance. This finding showed that herbivore resistant plants can also support parasitoids, and therefore that the use of resistant plants need not necessarily impede biocontrol services.

This findings emphasizes that testing plant resistance in a tritrophic context is crucial for its synergistic application with pollinators and biocontrol agents, and that resistant plant genotypes therefore need to be selected carefully. Thus, this work underlines the importance and urgency to routinely integrate natural enemies into the evaluation of resistance in crop improvement programs.

To fulfil growing policy and public demand, worldwide crop production is shifting from depending heavily on pesticides to a more sustainable integrated pest management. Yet, for successful and optimal implementation of IPM it is important to ensure synergistic interactions between of crop plants, pests, and mutualists (e.g. pollinators and natural enemies). Thus, as this work demonstrates, deeper insight into trophic interactions surrounding the crop is a crucial stepping stone.

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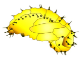

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Figure 14.
Drawing: Daniela Weber