Plant sex effects on biotic interactions in dioecious willow

Plant sex-specific effects on interactions between *Salix viminalis* and its herbivores, pollinators and fungal disease

Kim K. Moritz
NJ Faculty
Department of Ecology
Uppsala

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Cover: Interactions among studied species in a tritrophic setting.
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Abstract

Sex in dioecious plants affects interactions between plant individuals and the organisms consuming them. Plant-herbivore interactions are affected by plant sex across many plant families, and plant-pathogen infections can be strongly sex-biased. Non-antagonistic consumers of plant materials can also be affected by sex; pollinators often prefer plants of either plant sex. Still, much remains to be investigated of the mechanisms underlying these plant sex effects. In addition, most knowledge of plant-sex biased interactions focus on plant individuals and much less is known of how these effects scale up to groups of plants or populations. Finally, applications of plant sex effects in agricultural systems are lacking.

I established a large-scale field experiment using plots of a dioecious bioenergy crop, the willow *Salix viminalis*, to investigate what effects individual plant sex, and groups of males, females or sex-mixes, have on consumer organisms. I measured abundances of the most serious insect pest (*Phratora vulgatissima*), and one of its main predators (*Anthocoris nemorum*). Furthermore, I measured damage caused by roe deer (*Capreolus capreolus*) and infection of the most serious pathogens (*Melampsora spp.*) affecting *S. viminalis*. Finally, I measured effects of *S. viminalis* sex on pollinator visitation and on pollination in nearby strawberry. I performed laboratory-and greenhouse based controlled experiments to investigate mechanisms underlying the consumer interactions.

I found that female plants were more seriously affected by the pathogens but that infections were unaffected by plant sex ratio of plots. Neither the pest nor its predator was affected by plant sex in the field experiments despite effects on some life history traits in laboratory experiments. Roe deer browsing increased in mixed-sex plots, but roe deer did not discriminate between male and female individual plants. Pollinators more often visited male plants but mixed-sex *S. viminalis* still increased nearby strawberry fruit production.

Conclusions of this thesis are that plant-consumer species pair specificity matter for plant sex effects and that effects from individual plant interactions may differ from effects on interactions between plant groups and consumers. A better understanding of underlying mechanisms may be key to understanding how plant sex effects can be predicted and generalized. I also conclude that it is likely better to use male, than female, genotypes for *S. viminalis* plantations.

Keywords: Dioecy, Plant sex, Herbivory, Secondary metabolites, Fungal disease, Sex-bias, Pollination, Salicaceae, Ecosystem services

Author's address: Kim Moritz, SLU, Department of Ecology,
P.O. Box 7044, 750 07 Uppsala, Sweden
E-mail: kim.moritz@hotmail.com

Sammanfattning

Kön hos tvåbyggande (dioika) växter påverkar interaktioner mellan växtindivider och organismer som konsumerar dem. Interaktioner mellan växter och växtätare har visats påverkas av växtkön i flera växtfamiljer och infektioner av svampsjukdomar kan vara könsskeva. Även ickeantagonistiska konsumenter av växtmaterial kan påverkas av växtkön; pollinatörer föredrar ofta att besöka växter av endera könet. Ändå finns mycket kvar att undersöka gällande mekanismerna som leder till växtkönseffekter på interaktioner. Dessutom rör den kunskap som finns framförallt interaktioner hos individuella växter och mycket mindre är känt om hur dessa effekter påverkar grupper av växter eller populationer. Slutligen kvarstår tillämpning av den kunskap som finns om könsskeva interaktioner, i lantbruket.

Jag utförde ett storskaligt fältexperiment med en tvåbyggande bioenergigröda, videt *Salix viminalis*, för att undersöka vilka effekter individens kön och hela grupper av han- eller honväxter, eller en mix därav, hade på växtens konsumentorganismer. Jag mätte abundanser av den allvarligaste insektsskadegöraren (*Phratora vulgatissima*) och en av dess viktigaste naturliga fiender (*Anthocoris nemorum*). Jag uppmätte även skada orsakad av rådjur (*Capreolus capreolus*) och infektioner av de allvarligaste svamppatogenerna (*Melampsora spp.*) som drabbar Salixodlingar. Slutligen uppmätte jag effekter av växtkön på pollinatörsbesök och på pollinering av närliggande smultronplantor (*Fragaria vesca*). För att undersöka mekanismerna bakom interaktioner mellan växten och dess konsumenterutförde jag en serie kontrollerade experiment i labb och växthus.

Jag fann att honväxter hade allvarligare angrepp av svampjukdomen, men också att angreppen var opåverkade av konstellationer av hon- och hanväxter. Varken skadeinsekten eller dess naturliga fiende påverkades av växtkön i fältexperimentet, trots att vissa effekter kunde urskiljas i växthusexperimenten. Rådjursbetning ökade starkt i fält där växtkönen blandades, men ingen skillnad fanns i betning av han- och honväxter. Pollinatörer besökte hanväxter oftare än honväxter, men en blanding av han- och honväxter av ledde till större skördar från närliggande smultronplantor.

De huvudsakliga slutsatserna är att artparen som ingår i växt-konsumentinteraktioner kan ha artspecifika könseffekter, att effekterna från växtindividers kön kan skilja sig från effekter från könssammansättning av grupper av växter och att en bättre förståelse av underliggande mekanismer kan vara nyckeln till hur växtkönseffekter kan förstås och generaliseras. Vidare drar jag slutsatsen att hanväxter bör användas i odlingar.

Dedication

Tillägnad min familj.

Less is more... how can that be? It's impossible... more is more.
Yngwie Malmsteen

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 Moritz KK, Björkman C, Parachnowitsch AL, Stenberg JA. Effects of plant sex for biological control of insect herbivores in a Short Rotation Coppice willow (submitted manuscript).

II Moritz KK, Parachnowitsch AL, Julkunen-Tiitto R, Björkman C, Ayres MP, Stenberg JA (2016). Roe deer prefer mixed sex willow stands over monosexual stands but do not discriminate between male and female plants (manuscript).

III Moritz KK, Björkman C, Parachnowitsch AL, Stenberg JA (2016). Female Salix viminalis are more severely infected by Melampsora spp. but neither sex experiences associational effects. *Ecology and Evolution* 6(4), 1154-1162.

IV Moritz KK, Parachnowitsch AL, Björkman C, Stenberg JA. Associational legacy effects of intercropped Salix plant sex on strawberry yield driven by pollination (manuscript).

The contribution of Kim K. Moritz to the papers included in this thesis was as follows:

I Planned and performed the experiments and statistical analysis, and wrote the manuscript, with the help of Johan A. Stenberg, Christer Björkman and Amy L. Parachnowitsch.

II Planned and performed the experiments and statistical analysis, and wrote the manuscript, with the help of Johan A. Stenberg, Christer Björkman and Amy L. Parachnowitsch.

III Planned and performed the experiments and statistical analysis, and wrote the manuscript, with the help of Johan A. Stenberg, Christer Björkman, Amy L. Parachnowitsch, Riitta Julkunen-Tiitto and Matthew P. Ayres.

IV Planned and performed the experiments and statistical analysis, and wrote the manuscript, with the help of Johan A. Stenberg, Christer Björkman and Amy L. Parachnowitsch.

Abbreviations

SRC	Short Rotation Coppice
IPM	Integrated Pest Management
DLH	The Detoxification Limitation Hypothesis
d.f.	Degrees of freedom (statistical testing)
CCA	Canonical Correspondence Analysis

1 Introduction

This thesis elaborates on what is known, and what is still in need of further investigation, regarding trophic interactions between consumer organisms and dioecious plants.

1.1 Sex-biased trophic interactions

1.1.1 Dioecious plants and trophic interactions

Dioecious plant species, i.e. species with separate male and female individuals, constitute ~5% of all plants (Renner 2014). They are present in a wide variety of habitat types and many of them are of economic importance. For example, *Pistacia* spp., *Cannabis* spp., *Spinacia oleracea* and *Humulus lupulus* are commercially grown on several continents. Among dioecious plants of importance are also the Salicaceae species. Salicaceae is the family of plants to which willows (*Salix* spp.) and poplars (*Populus* spp.) belong. Members of this family are present on all continents except for Antarctica and some have been cultivated for various purposes, including forestry, garden decoration, basket weaving and bioenergy production.

All types of interactions between dioecious plants and consumer organisms, such as herbivory (Boecklen et al. 1994), pathogeny (Vega-Frutis et al. 2013) and pollination (Elmqvist et al. 1988), can be biased towards either males or females. Several sexually dimorphic plant traits, for example traits related to biochemical composition or structural defences, have been proposed as explanatory of sexually biased interactions (e.g. Ågren et al. 1999). This necessitates study of effects of each trait type for a fuller understanding of effects. These studies should also include effects on different types of consumer organisms interacting with male and female plants of the same plant species. Different types of consumer organisms can be expected to be affected by different plant traits. Depending on the type of plant-consumer interaction, *e.g.* the type of tissue that is consumed, timing of interaction events and variation in impacts of interactions on the plant, a study of all biased interactions may give us insight into mechanisms behind sex-biased interactions.

1.1.2 Physiological and morphological differentiation of plant sexes as adaptations to biotic interactions, and their relation to ecological interactions

Dioecy is polyphyletic in flowering plants, and plant families may contain species that are monoecious and dioecious (including gynodioecious and androdioecious). Within dioecious plants, male and female plants often differ in several vegetative traits, such as morphology, secondary chemistry and phenology, and in reproductive traits such as nectar production, flower longevity and costs of flowering. The origin of this intersexual diversity has been at least partly ascribed to ecological interactions such as co-evolution with mutualistic and antagonistic animals and fungi, often in relation to defence, growth and reproductive costs. Lloyd & Webb (1977) proposed that allocation of limited resources was key to understanding sexual dimorphism. They argued that because female plants often have higher costs associated with reproduction, the resource trade-off between growth and defence is more balanced towards the latter than for male plants, whose costs of reproduction is lower (Figure 1). There is indeed supporting evidence for females in many dioecious plant species spending more resources on reproduction (Obeso 2002), and female plants allocate resources to seed development in addition to gamete production, which takes place after flowering (Goldman & Willson 1986). Male plants of many dioecious species also grow faster or larger (reviewed by Obeso 2002). There are however exceptions from these patterns in resource allocation (e.g. Quinn 1991), defences (e.g. Bañuelos et al. 2004) and growth (Ueno & Seiwa 2003), indicating that resource allocation patterns alone cannot explain sexual dimorphism (Obeso 2002; Avila-Sakar & Romanow 2012). Other mechanisms affecting sexual dimorphism in plant defences against antagonists may for example include seed predation effects without regard to resource costs. Differentiation of traits of males and females might also have occurred because of intersexual differences in ecological optimal niches, which could explain spatial segregation of plant sexes (Meagher 1980). Additionally, trait plasticity may be differentiated between the plant sexes, leading to sexual dimorphism (Delph & Bell 2008).

1.2 Effects of plant sex on dioecious plants

1.2.1 Herbivory

In dioecious plants, herbivores may prefer plants of either sex, most commonly males (Cornelissen & Stiling 2005). Plant sex may be a very important trait for structuring herbivory and the possibility of utilizing plant sex for herbivory reduction should be investigated. A first step towards being able to choose plant cultivars that can decrease herbivory is to identify genetically based traits that influence herbivores. One strong candidate trait is plant sex because it can influence both herbivory and predation of herbivores on wild plants (Mooney et al. 2012; Kabir et al. 2014). Among traits that vary with plant sex are resistance-related traits (Bañuelos et al. 2004; Pollard & Briggs 1984; Boecklen et al. 1994), which likely explains a large part of the intersexual variation in herbivory. Male-biased herbivory in dioecious plants is more common than female-biased herbivory, strengthening the resource allocation-related hypothesis proposed by Lloyd and Webb (1977). However, female-biased herbivory and lack of sex-bias in herbivory are also common (Stiling & Cornelissen 2005), which suggests that resource allocation due to different reproductive costs does not provide a complete explanation of the mechanisms leading to sex-biases in herbivory.

The specialist *Phratora vulgatissima* is the most serious pest in *Salix* Short Rotation Coppice plantations. Because *P. vulgatissima* can be affected by host plant sex of a wild relative of *S. viminalis, S. cinerea* (Kabir et al. 2014), utilizing plant sex for herbivory reduction in commercial SRC should be investigated. I studied the effects of *S. viminalis* sex on *P. vulgatissima* abundances, herbivory, development and reproduction, to establish what effects plant sex has and what the underlying mechanisms are (Paper I).

Herbivores should balance nutrition and avoidance of harmful substances (Marsh et al. 2006). Generalist herbivores may choose to adjust consumption of plant species or genotypes based on plant secondary metabolites. For example, if generalists are more sensitive to concentrations or profiles of secondary metabolite content of digested plants, they may choose to browse on less well-defended plants. Alternatively, they may prefer to mix intake of plants to reduce levels of one or several compounds. Such a strategy can be expected under the detoxification limitation hypothesis (Freeland & Janzen 1974; Dearing & Cork 1999). The detoxification limitation hypothesis proposes that limitations in digestion of toxic compounds cause generalist herbivores to diversify their diet because they more easily cope with low concentrations of several toxins than high levels of any toxin (reviewed by Marsh et al. 2006). Monitoring of detoxification of both mammals (Dearing & Cork 1999) and other animals (Miura & Ohsaki 2004; Unsicker et al. 2008; Pankoke et al. 2012; Sotka & Gantz 2013) have shown that adding and mixing toxins lead to effects expected under the detoxification limitation hypothesis, so there is good reason to investigate what effects mixing different plant types may have on ecological interactions between plants and generalist herbivores.

Most previous studies of detoxification limitation focused on variation among plant species although all genetically based sources of variation between plant secondary metabolites should lead to the same type of effects. For example, plant sex is a source of variation in plant secondary metabolites in many species (e.g. Nybakken & Julkunen-Tiitto 2013). Plant sex ratios in populations can vary strongly among species (Barrett et al. 2010), and strongly sex-biased stands can be found of clonally reproducing plants (Alliende & Harper 1989). Although male-biased sex ratios are more common, both biases are common, as are populations with even sex ratios (Barrett et al. 2010). Causes for sex-biases are often genetically caused (Alström-Rapaport et al. 1997; Åhman 1997) or caused by abiotic factors (Petry et al. 2016), but may also be caused by intersexual differences in mortality. One factor leading to mortality-caused biases might be herbivory (Elmqvist & Gardfjell 1988). I therefore chose to evaluate the detoxification limitation hypothesis for plant sex as a source of variation. At the same time, I tested whether individual plant sex had any effects on roe deer browsing (Paper II).

A major generalist browser in *Salix* short SRC plantations is roe deer (*Capreolus capreolus*). Major differences between the types of herbivory exerted by *e.g. P. vulgatissima* and roe deer include scale and specialization, because roe deer may move for larger distances in a day than *P. vulgatissima* do during its lifetime and because roe deer are generalist browsers. Therefore choices that roe deer make may be more about groups of plants than about individual plants, which may have implications for browsing patterns. If, for example, roe deer prefer female plants they may prefer browsing groups of plants that have female-biased sex ratios.

1.2.2 Biological control agents

While plant sex effects on herbivores are widely studied for many dioecious species, much less is known about plant sex effects on natural enemies of herbivores (but see Mooney et al. 2012). There are however good reasons to expect such effects, especially for omnivorous herbivores that depend on host plant quality. There is evidence that genetically based plants traits can influence life history traits of both omnivorous predators and parasitoids, both directly (Wäckers et al. 2005) and indirectly (Fortuna et al. 2014). Insect omnivorous predators can be dependent on host plant quality for survival, foraging decisions (e.g. willingness to predate on animal prey *versus* consume plant material or choice of host plant), development, body size and reproduction. Plant traits that could influence omnivorous predators include nutritional quality of plant tissues, availability of floral and extrafloral nectar, structural defences and secondary metabolites. However, large-scale field studies of effects of plants traits in general, and of plant sex in particular, is lacking and it is unclear how effects that have been investigated are expressed under field conditions. Currently, the focus of research aiming at improving biological control is on improving conditions for promoting natural enemies of herbivores, by utilising companion plants (Bickerton & Hamilton 2012), flower strips (Tschumi et al. 2015) and other intercropping techniques (Landis et al. 2000). The mechanisms motivating these management options often include food and habitat provisioning for biological control agents such as omnivorous predators. As evidence grows that plant varieties and other types of genetically based phenotypic variation can be a source of effects on natural enemies of herbivores in agriculture (e.g. Chen, Gols & Benrey 2015), there is reason to investigate usage of crop genetic resources for biological control.

Utilization of host plant effects in a tritrophic system with the ultimate goal of reducing herbivory, requires positive effects on predation outweighing any effects on total herbivory. Thus effects on the two upper trophic levels must be simultaneously evaluated. Simpler cases are those where predators benefit from a plant trait but the herbivore experience no or negative effects. If, however, both herbivores and their predators receive benefits of a certain trait, the effects on the predators must be larger in order for total herbivory to be reduced. Conversely, if both the effects on the herbivore and the predators are negative, the negative effects on the herbivore must lead to less herbivory and should not provide a too great release from predation. This is particularly important if the herbivore species is a potentially outbreaking species and the predator population can efficiently prevent the herbivore population from outbreaking.

Plant sex can have effects on natural enemies of herbivores (Mooney et al. 2012; Kabir et al. 2014) and vary in how well they provision them with food (Bañuelos et al. 2004; Boecklen et al. 1994; Bullock 1984; Pollard & Briggs 1984), and should therefore be investigated as a source of biocontrol improvement. One potential source of intersexual variation is that of resource allocation differences due to different reproductive costs (Lloyd & Webb 1977). Increased allocation toward predator-rewarding traits would enhance indirect defence through biological control. A consequence, under the hypothesis of reproductive costs leading to different allocation patterns to defence, may therefore be that female plants allocate more resources to traits that reward predators of antagonists. There could also be a difference in how herbivores and their predators are affected by nutritional variation, because herbivores are generally more limited by nitrogen sources such as amino acids (Mattson 1980). Plant tissues often vary in nutritional value, and for example flower-produced food, which can differ between sexes, consumed by both herbivores and omnivores, can therefore be expected to affect consumers differently.

Effects of plant traits on predators of herbivores are potentially powerful tools for biological control, and should be evaluated further as a means to improve Integrated Pest Management (IPM). Because plant sex effects on herbivores are well-studied and there are examples of effects on predators, plant sex is a candidate for testing manipulation of biocontrol through plant traits. In this thesis (Paper I), I investigated whether choosing plant sex for SRC plantations of *S. viminalis* can provide improved biological control of *Phratora* *vulgatissima* by promoting its main predator, the omnivorous *A. nemorum* (Figure 1).

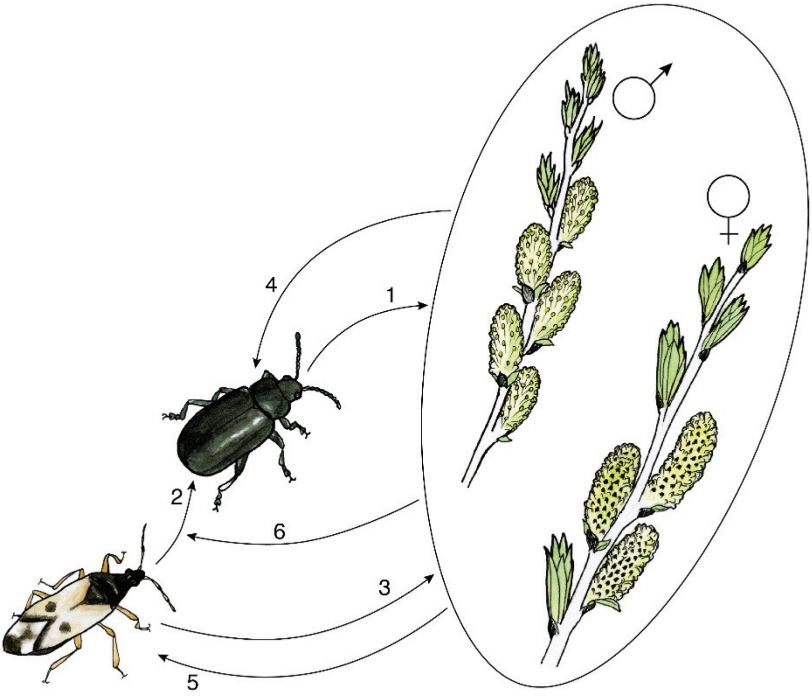

Figure 1. Tritrophic interactions in the studied system. 1: herbivory by the herbivore, 2: predation 3: herbivory by the omnivore, 4: plant sex effects on the herbivore and its herbivory, 5: plant sex effects on the omnivore and its herbivory, 6: plant sex effects.

1.2.3 Plant sex effects on pathogens

Host plant sex can be expected to affect pathogens in similar ways as it affects herbivores. That is, plants of the sex that is better defended can be expected to be less severely affected by pathogens. Fungal pathogens are the best-studied among plant pathogens, both in general and with regards to plant sex. Fungal pathogen infection in several dioecious species are sex-biased (e.g. Åhman 1997; Chandra & Huff 2014), however many of the findings are relatively recent and the subject is in need of summary and review.

Most of our current understanding of plant sex effects on fungal infection concerns plants at an individual plant level, while effects at plant population or group level from diversity are common. Neighbourhood composition of plant genotype or species can affect a focal plant's interactions with other organisms ('Associational effects', c.f. Underwood et al. 2014). Associational effects on ecological interactions have previously been found for diversity, or composition, of plant species (Parachnowitsch et al. 2014), genotypes (Parker et al. 2010) or genes (Zhu et al. 2000), but effects from other types of variation are largely unexplored. Plant sex can have effects on interactions such as fungal pathogen infection, and pathogen infection can be subject to associational effects of different types of plant diversity. Therefore, associational plant sex effects can potentially be an important part in understanding plant sex-biased pathogen infections. Pathogen infection in plants can be subject to associational effects because disease spread can be hampered by a reduced likelihood that plants neighbouring an infected plant have the same pathotype susceptibility (Browning & Frey 1969; Leonard 1969). Supporting evidence for associational effects on fungal disease have been provided for cultivar mixes (Zhu et al. 2000) and for clone mixes (Begley et al. 2009), but no experimental approach has previously been taken to investigate whether associational effects can be found for mixing plants of different sex. Such effects could be expected if plant sexes are differently affected, because plant sex is mostly genetically based and there is plentiful evidence for genetic variation at different levels causing associational effects. Previous studies of plant sex effects on interactions with fungal pathogens have mostly studied effects for individual plants, and many of these have been made in mixed-sex plantations or stands. Effects of sexual monocultures have therefore been largely ignored, despite plantations and natural stands of dioecious plants often being monosexual.

There is currently no consensus on mechanisms driving plant sex biased fungal infection. One explanation for female-biased infection could be resource limitation trade-offs and differences in life-history traits (Rolff 2002; Vega-Frutis et al. 2013), but that only offers an explanation for male-biased pathogen infections, which are not the majority of cases.

Melampsora spp. infections in the Salicaceae family are among the best investigated fungal pathogens infecting dioecious plants (e.g. Lascoux et al. 1996; Mccracken et al. 2000; Pei et al. 1993). In *S. viminalis*, female plants have previously been recorded to have female-biased infections in one year and no sex effects on infection in another year (Åhman 1997). In this thesis I investigated whether female monosexual plantations of *S. viminalis* are more severely infected, and whether mixed sex plots are less severely infected by *Melampsora* spp., than monosexual plots (Paper III). In addition, I evaluated whether plant sex on an individual level affected infection severity. To further investigate *S. viminalis* sex effects on *Melampsora* spp. infection, I surveyed previous studies that compared clones and re-investigated the results using information on the sex of used clones. I also surveyed published studies of fungal pathogen infection in dioecious plants to determine whether plant sex-biased infections in either direction is more common than the other. In line with previous studies on *Salix viminalis* susceptibility to *Melampsora* spp. I expected females to be more severely infected. I therefore also expected female plantations to be more severely affected, and an associational effect of monosexual female plots increasing the infection. In contrast, I expected a mix of male and female plants to decrease infection severity. Finally, I tested whether clones responded differently to neighborhood sex ratios. I expected the survey results from previous studies on *S. viminalis* and *Melampsora* spp. would reflect my field results. However, I expected the general pattern in dioecious plants to be male-biased infections, based on resource allocation theory.

1.2.4 Plant sex effects on pollinators

Common differences between male and female plants of dioecious species regard flowers. These differences may in turn influence pollinators. Because sex of dioecious plants can influence pollinator behaviour (e.g. Vaughton & Ramsey 1998), there may be potential for utilizing plant sex effects to promote important services that pollinators provide agriculture. Pollen is an important source of nutrition for many pollinator species (Reddersen 2001), and may be of great value for sustaining pollinator populations. Flowers of male plants often provide visitors with pollen in addition to nectar and are often more frequently visited than flowers of female plants (Ostaff et al. 2015). From an applied perspective, it may thus be beneficial to choose to include male plants of dioecious species if grown close to pollinator-dependent crops if a larger pollinator community can be sustained. However, it is not clear that planting male plants only would have desired effects. For example, it is also possible that male plants of a plant species that is highly attractive to pollinators instead compete with crops with overlapping flowering times.

While a highly attractive plant species may compete with a pollen-limited crop if flowering times of the two plant species overlap they may provide benefits if they are instead flowering in sequence. Field experiments in which flowers of plant species have been removed in different sequences among treatments have demonstrated that one plant species can inherit attracted pollinators of another (Ogilvie & Thomson 2016). If plant sex effects of an early-flowering dioecious plant species exist, they may therefore provide legacy effects on pollination of subsequently flowering species.

Mixes of male and female plants may be better at promoting pollinator communities than monosexual plantations, if female nectar is of higher quality than male nectar but only male plants provide pollen. For example, females of *S. myrsinifolia* produce nectar with higher sugar concentrations than males and total sugar production per flower can be higher in *S. caprea* and *S. cinerea* (Kay 1985), although *S. caprea* males and female flowers can also produce similar quantities of sugar (Dötterl et al. 2014). Females producing more nutritious nectar than males may for example be an evolutionary adaptation that compensates for lacking pollen as a reward for visiting pollinators.

Plant sex differences in willows may be important for pollinator communities and can therefore potentially be utilized to promote pollinator services in agricultural settings. *Salix* spp. SRC plantations most often occur in agricultural landscapes and the floral resources that they provide to pollinators can be of great value for pollinators, especially early in spring (Reddersen 2001). *Salix viminalis* males produce pollen and nectar, while females produce nectar only. Female *Salix* spp. do in general produce nectar with more equal ratios of the three main sugars sucrose, glucose and fructose (Katoh et al. 1985), which can be preferred by bumblebees (Wykes 1952). Because of plant sex differences in pollinator rewards in *Salix* spp., effects of plant sex on pollinators and pollination on nearby crops should be investigated. I therefore investigated whether pollinator visitation, rewards and pollination of a nearby pollen-limited crop, *F. vesca*, were affected by sex of *S. viminalis* individuals, and by monosexual male or female plantations or a mix of males and females (Paper IV).

2 Aims

In this thesis, I develop our understanding of how dioecy in plants affects interactions with consumer organisms using *Salix viminalis* as a model. The consumer organisms represent different types of consumers, belonging to different guilds, and different organism groups; a herbivorous leaf beetle (*Phratora vulgatissima*), its natural enemy the omnivore *Anthocoris nemorum*, a mammalian browser (*Capreolus capreolus*) and pathogenic fungi (*Melampsora* spp.).

The specific goals with this thesis were to:

1. Investigate what effects plant sex of individuals, and plant sex composition of groups of plants, have on herbivory and pathogeny (Paper I, II, III).
2. Explore how the plant trait-dependent mechanisms through which consumer interactions with dioecious plants are affected by plant sex and plant sex composition, and affect interactions with consumer organisms (Paper I, II, III, IV).
3. Examine whether biological control of herbivores could be enhanced in agricultural settings by including plant sex selection as a management practice (Paper I).
4. Evaluate suitability of male and female plants, and of a mix thereof, as food source for pollinators (Paper IV).

3 Materials and methods

To evaluate whether plant sex affect herbivory, predation of herbivores, fungal pathogens and pollination in *Salix viminalis* individuals and stands, I set up a large-scale field site in which my hypotheses could be evaluated based on derived predictions. Furthermore, to investigate mechanisms behind patterns that would be detected in the field, I performed separate experiments in greenhouses and labs using the same, or mostly the same, *S. viminalis* clones. All experiments apart from the phenolic analyses were performed in Uppsala, Sweden, between 2013 and 2016.

3.1 Species descriptions

3.1.1 Plants

Salix viminalis L., the common osier, is a dioecious willow that has been found in Sweden since the 1700's (Hylander 1971). Male individuals produce both nectar and pollen, whereas female plants produce nectar, but no pollen. It grows as a shrub or a tree and one plant often produces several shoots. *S. viminalis* is mainly used for bioenergy production. Because it is an economically low-yielding crop per area unit, reduction of herbivory and fungal disease by means of pesticides has been deemed economically and environmentally unsustainable (Stenberg et al. 2010). Therefore, breeding genotypes that have a genetically based higher resistance, lower palatability to herbivores or lower pathogen susceptibility could provide better means of reducing biomass losses and enhancing productivity and economic viability.

Plantations of *S. viminalis* most often consists of one single clone (Reddersen 2001), and are therefore strictly monosexual, and abundances of wild populations of different of dioecious plants are often sex biased (Barrett et al. 2010). These patterns in spatial segregation of the sexes makes *S. viminalis* a good study system for ecological effects of plant sex of individuals, and for ecological associational plant sex effects on consumer organisms.

Fragaria vesca L., woodland strawberry, is a perennial plant that is native to Sweden and occurs throughout Europe. It is a perennial plant. Cultivars of *F. vesca* are commercially grown for its fruits. If its flowers are not fully pollinated, they can be aborted, or produce deformed fruit (Klatt et al. 2013).

3.1.2 Insect herbivore and omnivore

Phratora vulgatissima L. (Chrysomelidae) is native to Sweden. It is mostly black with blue-green metallic nuances. In the studied area, it is univoltine (Björkman & Eklund 2006). The first (overwintering) generation of adult beetles in a season appear on Salicaceae species in May-June and the new generation appear approximately in early August. All *P. vulgatissima* used in my laboratory and greenhouse experiments were taken from laboratory cultures originally collected in Hågadalen (Latitude: 59°49', Longitude 17°34'), outside of Uppsala, Sweden.

Anthocoris nemorum L. (Anthocoridae), the common flowerbug, is native to Sweden and appears in the field around the same time as *S. viminalis* leafing, April-May. It undergoes multiple overlapping generation shifts throughout a season and can be found until October in the study area. It is a common omnivore occurring on *Salicaceae* species in the beginning of the season, when these plants' nectar provide an early food source, but its effects on the plants themselves are negligible (Lauenstein 1979). *Salix* spp. genotype has effects on *A. nemorum* survival and predation efficiency (Stenberg et al. 2011). Later in the season, it is also commonly found on *Urtica dioica*, and the flower bugs used in my experiments were mostly collected on *Salix* spp. and *U. dioica*. It is a common predator of *P. vulgatissima*, consuming eggs and early-instar larvae. *Phratora vulgatissima* eggs that have been consumed by *A. nemorum* are clearly distinguishable from intact eggs (Stenberg et al. 2011).

3.1.3 Generalist mammalian herbivore

Roe deer (*Capreolus capreolus* L.) is a generalist ungulate that can cause serious damage to both wild *Salix* spp. plants and *Salix* short rotation coppice (Bergstrom & Guillet 2002).

3.1.4 Fungal pathogen

Melampsora spp. are the most serious pathogens in *Salix* SRC plantations (Ramstedt 1999; Pei et al. 2004), causing up to 40% biomass losses (Parker et al. 1993; as reported by Pei et al. 2004). *Melampsora* spp. asexually produce multiple overlapping generations over the vegetative season of *S. viminalis*. Infected leaves are easily identified in late summer and autumn, by yellow to orange uredina mainly on undersides of leaves.

3.2 Field experiments

The field site was located east of Uppsala, Sweden (Latitude: 58°83', Longitude: 17°78', Figure 2).

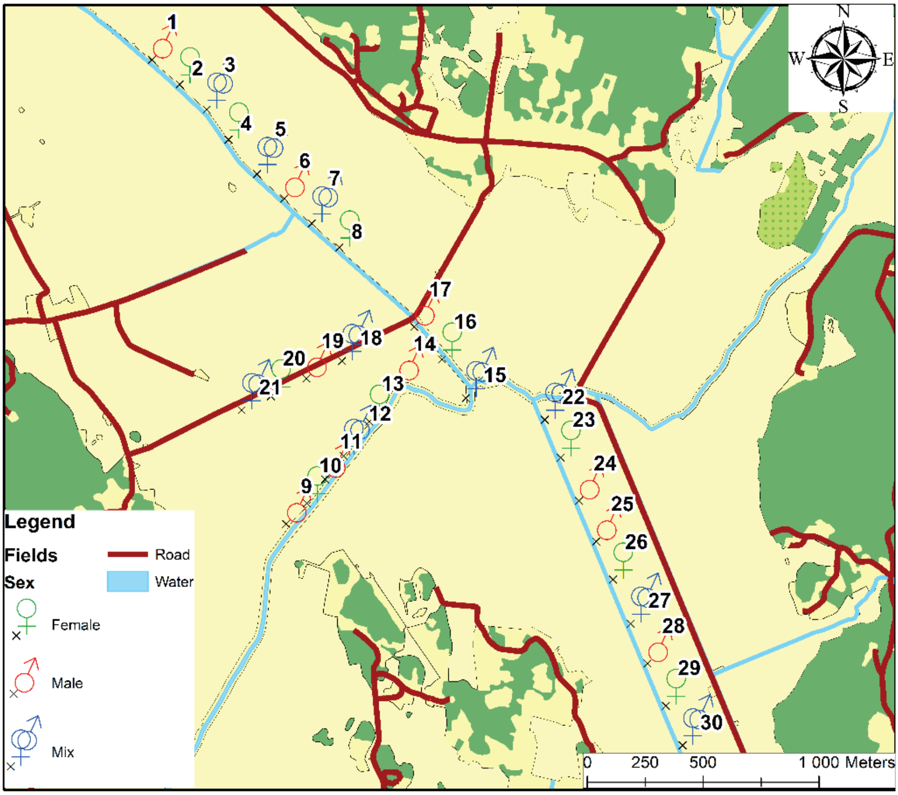

Figure 2. Map over field experiment, with male and female monosexual plots, and plots comprised of even mixes of male and female genotypes.

At the field site, I planted cuttings of 40 experimental clones (20 male and 20 female clones) in 30 plots. The experimental clones were originally collected from wild populations in different regions of Sweden and had been growing in experimental common gardens, “Clone archives”, south of Uppsala, Sweden (Latitude: 59°80, Longitude: 17°66) under harvest regimes similar to that of most commercial fields. I collected 20 cm long cuttings from the clone archives in March 2013 and stored them in -5^{o}C in a dark room until I planted them in May 2013. To exclude competition from other plants, I covered each plot area with a 10·10 m weed mat. Centred in the mat, I arranged a grid in which 40 *S. viminalis* cuttings were planted through holes cut in the weed mat. The plots had a nearly-quadratic shape, as cuttings were planted in six rows of six plants, followed by one row of four plants. I left 70 cm between neighbouring cuttings because this distance is used between rows of plants in commercial *S. viminalis* fields. The field site’s 30 plots were grouped into 10 spatial blocks of plants, each block containing one of each of three treatments; all male, all female or a mix of male and female clones. The intention with a division into spatial blocks was to counter any effects of spatial heterogeneity biasing results for either of the plot sex treatments. Within each monosexual (male or female) plot, two cuttings of each clone of the chosen sex were planted according to a plot-unique plan of randomized positions. Within the mixed sex treatment, male and female clones were randomly distributed over the plots such that each clone appeared equally many times in the total experiment, but only ten plants of each sex were used in each plot. To establish equal genotypic diversity among treatments, and thus not risking a diversity-effect masking plant sex effects, each clone present in a mixed sex plot was planted two times, as in the monosexual treatments. After the plantation was discovered it was discovered that some of the experimental clones were genetically identical or incorrectly sexed, and in May in 2014 I replaced those clones with new clones. Halfway in between the *S. viminalis* plants and plot (*i.e.* weed mat) edges on all four sides, I planted woodland strawberry (*F. vesca*) plants of the commercial variety "Rügen" in quadratic-shaped groups of nine. Within the strawberry groups, distances between plants were approximately 20 cm.

3.2.1 Insect herbivore and insect omnivore abundances

To estimate differences in relative abundances of *P. vulgatissima* and *A. nemorum* between plant sexes and among plot sex treatments (Paper I), I sampled insects in the field experiment once in 2014 (July), three times in 2015 (June, July, August) and once in 2014 (July). On each plant in the field experiment, I visually scanned a randomly selected shoot and recorded the number of *P. vulgatissima* and *A. nemorum*.

3.2.2 Mammalian herbivory

Over the 2013 field season, the *S. viminalis* plantations were subject to heavy roe deer herbivory. All *S. viminalis* plants in all experimental plots were subject to top browsing (Figure 3), and most plants only consisted of one shoot. I therefore chose to measure inverse plant heights as a proxy for herbivory. I measured each plant using a measuring tape from the ground up to the most peripheral shoot tip (Paper II).

Figure 3. A *Salix viminalis* plant in the field experiment after roe deer browsing.

3.2.3 Fungal pathogen infection

In October in 2014, I recorded leaf rust severity in the field experiment in order to investigate plant sex effects, and plot sex treatment effects, on severity of *Melempsora* spp. severity (Paper III). In September, I visually scored a randomly selected shoot from each of the by then 1060 surviving plants according to a 7-level descriptive ordinal scale (c.f. Table 2 in Pei et al. 2008). To investigate effects that leaf area may have on infection severity, I measured leaf length and width in late July 2015 and calculated the leaf areas as areas of ellipses (area = π · width · length).

3.2.4 Pollinator visitation and strawberry fruit production

To test for plot sex treatment effects on pollination and strawberry fruit yield, I observed visitation and measured strawberry fruit production in the experimental plots after the *S. viminalis* plants had reached reproductive maturity (Paper IV). In July 2015, I counted strawberry fruits of one randomly selected *F. vesca* plant in each plot. To investigate whether the flowers of the plant were fully pollinated, I furthermore counted the number of malformations on a randomly selected berry. Malformations occur on fruits from flowers that have received some but not full pollination. Because I wanted to test for a relationship between malformation number and number of strawberry fruits, I counted both on the same plant. In 2016, I observed number of hoverfly and bee visitors over 10 minutes for each group of 9 strawberry plants in the experiment in July. Furthermore, I enclosed two randomly selected *F. vesca* plants in one 9-plant group per plot in perforated plastic bags to exclude pollinators. In one of the bagged plants per plot, I hand-pollinated the open flowers after one week. After three weeks, I removed the bags and counted the number of developing strawberry fruits produced by each plant. In addition, I counted fruits of the rest of the strawberry group's plants.

3.3 Laboratory experiments

3.3.1 Insect herbivory and predation experiments

To test for plant sex effects through separate mechanisms, I performed a series of manipulative bitrophic experiments with *P. vulgatissima* and *A. nemorum* on *S. viminalis*, and one tritrophic experiment where both insect species were involved (Paper I).

In order to test for preferred *S. viminalis* sex for *P. vulgatissima*, I tested 40 unique plant pair combinations of 19 male and 20 female clones. A pair of plants were placed in a net cage (base 40·40 cm, height 78 cm) and between the basal parts of the plant shoots, a Styrofoam bridge was placed. In each replication of the experiment, an adult *P. vulgatissima* was placed at the middle of the Styrofoam bridge, facing neither plant. After 30 minutes, I recorded the whereabouts of the insect (male plant, female plant or elsewhere).

I investigated whether there were differences in consumption rates of *P. vulgatissima* larvae on leaves of male and female *S. viminalis* by enclosing three neonate larvae on a single leaf in a plastic cup for 48 h in 20°C. I replicated the experiment over 19 of the genotypes that were present in the field study, of each sex. Each leaf was then photographed along with a paper with pre-drawn lines 0.5 cm apart and total consumed area was measured.

To test for adult *P. vulgatissima* oviposition and feeding preferences, I released 38 adult leaf beetles (19 beetles of each sex) into a net cage (base 60·160 cm, height 108 cm). Inside the cage, 19 *S. viminalis* plants per sex of different clones (the same clones as used in the field experiment) were placed in a randomized constellation. After ten days, I recorded the number of beetles present and number of eggs on each plant, and performed an image analysis as described above for *P.* vulgatissima larval *S.* viminalis leaf consumption.

To investigate *S. viminalis* sex effects on *P. vulgatissima* performance, I performed a non-choice feeding experiment from the first-instar larval stage to adult stage for the leaf beetles. I used 20 male and 20 female genotypes of *S. viminalis*, grown for six weeks in greenhouse conditions (18h light, 20^{o}C), as feed plants. I placed a larva on a detached leaf of a designated *S. viminalis* genotype each in separate transparent plastic vials (ø3 cm, 7 cm height). The vials were kept in a group with randomized positions inside a growth chamber (18 h light, 20^{o}C) for the duration of the experiment. Every second day until pupation, leaves were replaced with new leaves from the same genotype and larvae were moved between old and new leaves using a thin paintbrush. Completely formed pupae and newly emerged adult beetles were weighed using a Mettler Toledo MX5 microbalance (0-5.1 g ±1µg).

To investigate *S. viminalis* sex effects on *A. nemorum* performance, I performed a non-choice feeding experiment from the first-instar nymphal stage to adult stage for the flower bugs. I placed newly collected flowering branches of *S. viminalis* from the same clones and clone archives as used for cuttings in the field experiment in plastic mugs (ø9 cm, height 20 cm), with the upper part with leaves and catkins in one dry vial and the basal part in another mug of the same kind, but to two-thirds filled with water. No animal food was intentionally introduced, but small prey may have been available on branches. In the upper mug, one newly-hatched nymph hatched from an egg laid by a field-collected pregnant adult *A. nemorum* was placed on the plant shoot. The mugs were placed in a randomized pattern on a shelf in a climate chamber (18 h light, 20^{o}C) for the duration of the experiment. Developmental stage and survival of experimental *A. nemorum* individuals were recorded 15, 19 and 26 days after the start of the experiment.

To test for plant sex effects on predation efficiency, I performed a tritrophic experiment where I manipulated host plant sex. I grew 19 male and 20 female plants of the same experimental clones as used in the field experiment in 18h light, 20^{0}C, for 6 weeks on separate trays. I then covered each plant's shoots with a perforated plastic bag tightened around the basal part of the main shoot to prevent insects from escaping. I placed one pregnant female *P. vulgatissima* on each plant. Each experiment replicate started when the first *P. vulgatissima* eggs were laid. An adult *A. nemorum* bug was then added to the bagged plant. One week after the start of the experiment, I counted the surviving *P. vulgatissima* eggs and those that had been consumed by *A. nemorum*. I continued each replicate until the *A. nemorum* bug was dead and measured the survival time in the experiment.

3.3.2 Plant chemical analyses

To investigate intersexual differences in secondary metabolites, and to relate herbivory patterns to plant chemical defences, I mapped phenolic compounds present in leaves of the same clones as I used in the field experiment (Paper II). I planted five cuttings of each clone in soil that had been sterilized in a microwave oven at 900W, in flower pots. I grew the plants in randomized grids spread over six tables in a greenhouse (20^0C, 18 h Light) for 6 weeks and then harvested a mid-positioned mature leaf from each plant. I dried and milled the leaves. Extraction was performed using a homogenizer (Precellys) with 100 % methanol and analysed with High Throughput Liquid Chromatography as described in Nybakken et al. (2012).

To estimate nutritional values of *S.* viminalis clones of different sexes, I estimated nitrogen (N) content in leaves. To estimate N content in leaves, I used a chlorophyll meter (Minolta SPAD 502 Plus) on three plants per experimental clone, grown according to the same specifications as for the phenolic extraction experiment. The data from original clones that were discovered to be either incorrectly sexed or genetically identical to other experimental clones and thus removed from the field experiment were not used for statistical analyses.

3.3.3 Floral nectar analyses

To investigate whether male and female *S. viminalis* differ in nectar amount, I collected flowering twigs from 19 male and 20 female genotypes from the same clone archives as used for the field experiment cutting collections, in April in 2014 and 2015 (Paper IV). In the laboratory, I used 0.5µL microcapillary glass tubes (LAMAG 022.7725) to extract nectar from flowers. I collected nectar from ten flower nectaries per twig and measured the volume. For each genotype, the nectar measurement was repeated three times with separate twigs originating from different plants and averaged for that genotype. I also counted the number of flowers per catkin, catkins per randomly selected shoot for all experimental clones used in nectar analyses in 2015. I also compared nectar amounts for male and female *S. viminalis*, by using a 1 µL microcapillary glass tube (LAMAG 022.7726) to extract 0.5 µL of nectar from several flowers of a randomly selected catkin per clone. I then diluted the droplet with 10 µL deionized water. I blotted the diluted nectar onto the lens on a digital refractometer (MISCO Palm Abbe™ Digital Refractometer, Model No: PA201, range: 0.0 to 56.0 Brix, precision: +/-0.1 Brix, where 1 Brix corresponds to 1 g sucrose per 100g solution).

3.4 Statistical analyses

All statistical analyses were performed using different versions of R (R Development Core Team 2015), the earliest being 3.0.2. All generalized linear mixed models that had Poisson, binomial or quasi-Poisson or quasi-binomial error distributions were constructed using the package *lme4*. Unbalanced samples were compensated for by calculating type-II error distributions using the *car* package. Cumulative link mixed models of data on ordinal scales were constructed using the *ordinal* package. To perform random forest analyses, I used the *varSelRF* package. For canonical correspondence analysis, I used the *vegan* package. For plotting, I used the *base* and the *ggplot2* packages. To predict estimated means based on models for plotting the cumulative link mixed model, I used the *lsmeans* package. For other model types and functions than those mentioned above, the *base* package was used.

3.4.1 Field experiments

To test for *S. viminalis* sex effects on *P. vulgatissima* and *A. nemorum* adult abundances, I used separate generalized linear models for the two species. I used abundance on each plant as a response variable, plant sex and plot sex treatment as independent fixed factors. I used plot, block and date as independent random variables in a generalized linear mixed model with a Poisson error distribution and a log-link. In addition, for *P. vulgatissima* abundances, I included *S. viminalis* individual a random factor to account for repeated measurements.

To test for plant sex effects on roe deer herbivory, I constructed a linear mixed model with log-transformed *S. viminalis* height after browsing as a continuous linear response variable, plant sex and plot sex treatment as independent fixed factors and plot and block as independent random variables.

I tested for *S. viminalis* sex effects on *Melampsora* spp. infection severity using a cumulative link mixed model. In the model, I used the infection severity category of each plant as a response, plant sex and plot sex treatment as independent fixed factors, and plot and block as independent random factors.

To test for neighbour effects of plot sex treatment on strawberry fruit production on *F. vesca* plants close to *S. viminalis*, I used a generalized linear mixed model with Poisson error distribution and a log-link. I used 2016 strawberry fruit production as response variable, *S. viminalis* plot sex treatment as a fixed factor and block as a random factor. To test for pollinator exclusion effects on strawberry fruit production in the pollinator exclusion experiment, I used 2016 strawberry fruit production as a response variable, bagging and hand pollination as independent fixed factors and block and plot as independent random factors. For observations of pollinator visitations to strawberry groups close to *S. viminalis*, I used a generalized linear model with Poisson distribution and log link, in which number of visits to the group that resulted in contact with a flower were used as response variable, plot *S. viminalis* sex treatment as an independent fixed factor and block as an independent random factor.

3.4.2 Insect herbivory and predation experiments

To test for *P. vulgatissima* plant sex choice in cage experiments where a male and female *S. viminalis* plant were presented, I used a χ^2-test. To test for effects on larval feeding, I used consumed area as a response variable, and plant sex and number of surviving larvae at the end of the experiment, as factors in a linear regression model. For adult *P. vulgatissima* feeding differences between male and female *S. viminalis*, and for development time and weight I used t-tests. To test for *S. viminalis* sex effects on *P. vulgatissima* oviposition, I used a generalized linear model where egg number was used as a response variable and plant sex was used as a factor.

To test for plant sex effects on *A. nemorum* survival in the performance experiment, I used a generalized mixed model with a binomial error family and a log link, with survival as a response variable, plant sex a fixed factor and time as a random variable.

I tested for plant sex effects on predation rate of *A. nemorum* on *P. vulgatissima* eggs using a generalized linear model with a Poisson error distribution and a log link. I used number of eggs consumed as a response variable, plant sex as a fixed factor and total number of eggs as a random variable. To compare *A. nemorum* survival time in the tritrophic experiment, I used a t-test.

3.4.3 Plant chemical analyses

To analyse intersexual differences in *S. viminalis* leaf chemistry, I built a Random Forests model that was trained to separate male and female plants based on secondary metabolite content. I then predicted the sex of all plant samples, using the resulting model. To build the random forests model, I constructed 5000 trees using 200 bootstrap iterations (.632+). Error rates stabilized after around 50 bootstrap iterations. I calculated the model accuracy, which can be used as a measure of how informative a model is for the predicted variable. Furthermore, the model allowed us to determine which of the 19 secondary metabolites contained most information in concentrations between male and female plants.

Besides the Random Forests model, I also performed an analysis of plant sex differences in secondary chemistry using canonical correspondence analysis. I included concentrations of the secondary metabolites as response variables to plant sex. To test the model I used a permutation test with 9999 permutations. To estimate the effects of an even male-female mix of *S. viminalis* on leaf secondary metabolites, I used mean values of the first and second axes for each clone to calculate differences and calculated mean standard error and mean for vectors corresponding to random samples of clones, and compared them to corresponding vectors of all-male or all-female vectors (representing plots). I also compared total phenolic and lignan contents, and individual compounds, in male and female plants, using linear mixed models with log-transformed concentrations as response variables, sex as fixed factor and clone at random factors. To test for differences in N content, I used a linear mixed model to compare the SPAD values in male and female plants, with sex as fixed factor and clone as random factor.

3.4.4 Literature survey of plant sex differences in fungal pathogen infection

I performed two literature surveys to 1) examine whether there is a general pattern of sex-biased fungal pathogen infections of dioecious plants and 2) evaluate whether there is a typical pattern of sex-biased infections in previous studies of *S. viminalis* and *Melampsora* spp. (Paper III).

For the literature survey on sex-biased fungal infection of dioecious plants in general, I started with lists from two previous review articles (Ågren et al. 1999; Vega-Frutis et al. 2013) and added studies that I found searching Web of Science (Thomson Reuters 2015) for articles containing search keywords that related to plant dioecy and fungal pathogens, and references that were in turn found in those studies.

For the literature survey of *S. viminalis* sex effects on *Melampsora* spp. infection, I searched Web of Science (Thomson Reuters 2015) for articles containing the two species names, and included those in which at least two *S. viminalis* clones were used. In each study, I therefore noted the sex of the most severely infected clone without regards to statistical testing. To determine which clone belonged to which sex, I used information available at the Swedish University of Agricultural Sciences, Uppsala, Sweden, and at the National Willow Collection at Rothamstead Research, Harpenden, Great Britain (William Macalpine, personal communication).

3.4.5 Pollinator observations and floral resources

I used a generalized linear model with a Poisson distribution and log link to test for effects of *S. viminalis* sex on pollinator visitation in 2014 and 2015. Genotype and sex were used as fixed factors and visitation number as response variable.

I tested for intersexual differences in nectar volumes, sugar content and numbers of flowers using t-tests.

4 Results

4.1.1 Field experiment

There were no differences in *Phratora vulgatissima* abundance between male and female plants (χ2=0.77 P= 0.38, d.f.=1, Figure 4), and no differences between plot sex treatment (χ2=0.46, P=0.80, d.f.=2, Figure 4) over the 2014, 2015 and 2016 field sampling occasions (Paper I). Number of *P. vulgatissima* found ranged between 74 and 5 for the sampling occasions included in the model). The abundance of *P. vulgatissima* larvae did not differ between sexes (χ_2= 0.17, P= 0.68, d.f.=1) or plot sex treatment (χ_2= 0.12, P= 0.94, d.f.=2).

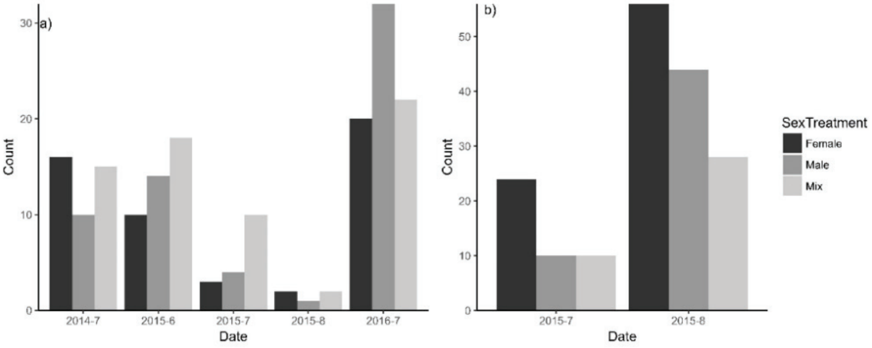

Figure 4. Total abundances in female, male and mixed sex plots of *Phratora vulgatissima* a) adults and b) larvae at different sampling occasions.

Abundances of *A. nemorum* also did not differ between sexes (χ_2= 0.60, P=0.44, d.f.=1) or plot sex treatments (χ_2= 2.11, P= 0.35, d.f.=1), in 2014, when *A. nemorum* were present in the field experiment (Paper I). In total, 23 individuals were found.

Salix viminalis heights in the field experiment differed among treatments after a growth season with roe deer herbivory (χ^2=7.88, P=0.02, d.f.=2, Figure 5), where mean heights of mixed-sex plots were taller than those in monosexual male, and monosexual female, plot treatments (Paper II). There was no effect of individual plant sex on height (χ2=0.01, P=0.95, d.f.=1). Correspondingly, plants in plots with mixed-sex treatments had lower survival. Monosexual female plots had the highest mean survival. In total, 140 plants had died at the time of recording, out of which 58 were males and 82 were females. There was no significant effect of individual plant sex on survival, however. Survival was positively correlated with plant height (r=0.36, p=0.0325, n=35).

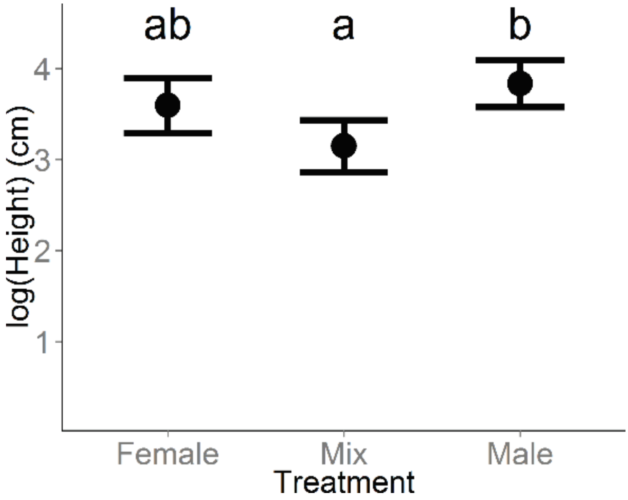

Figure 5. Heights of *Salix viminalis* in the field experiment after browsing by roe deer.

Melampsora spp. infection severity on *S.* viminalis in the field experiment was affected by individual plant sex (Paper III, z= -2.728, SE= 0.317, P= 0.006). Female plants had higher infection severity scores than male plants (Figure 6). Plot sex treatment, however, did not significantly affect infection severity (z =

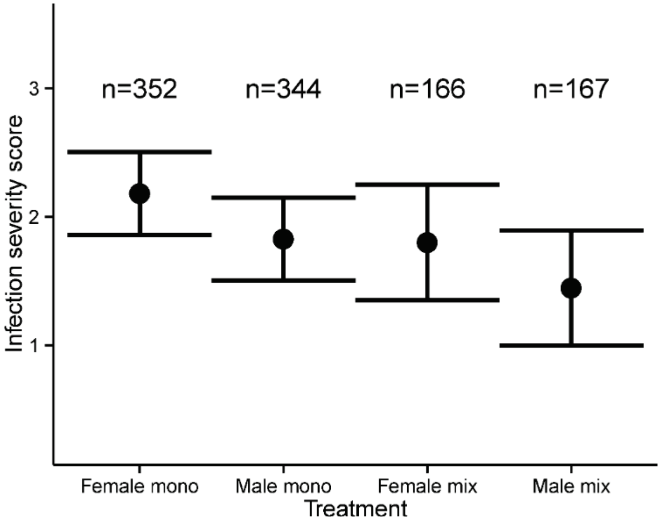

Figure 6. Infection severities of *Melampsora* spp. on *Salix viminalis* in the field experiment

0.705, SE = 1.320, P= 0.481), and there was no significant interaction between plant sex and plot sex treatment. At the time of recording leaf rust severity, survival of plants did not differ between sexes (d.f. = 1, z = 1.346, P= 0.178) or among treatments (d.f. = 1, z = 1.015, P = 0.310). There were no plant sex-related differences in leaf area (d.f. = 1, t = 0.56, P = 0.709) and phenology (d.f. = 1, t = 1.1, P = 0.471).

Strawberry fruit production was higher for *F. vesca* planted close to *S. viminalis* sex mixes in 2015 (d.f.=2, P=0.012 Figure 7), but not in 2016 (d.f.=2, P=0.997). There was an increased number of fruits produced when plants were hand-pollinated (d.f.=1, P<0.001), but no effect of pollinator exclusion by bagging (d.f.=1, P=0.80).

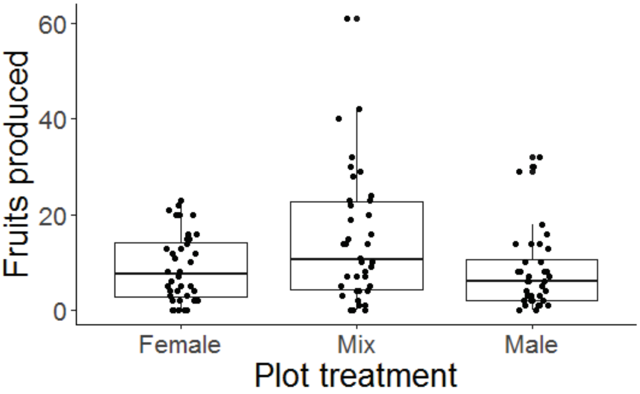

Figure 7. Numbers of strawberry fruits produced by plants adjacent to female, male or mixed-sex *Salix viminalis.* Data points are separated along the vertical axis. Boxplots denote medians, and upper and lower quartiles. Whiskers show 1.5 inter-quartile ranges.

4.1.2 Insect herbivory and predation experiments

Phratora vulgatissima were not found more frequently on male than on female *S. viminalis* (χ2=1.6, P=0.21, d.f.=1) in the single-insect host plant choice experiment (Paper I).

There was no difference in leaf area consumed by *P. vulgatissima* larvae between female and male *S. viminalis*. Adults that had been fed a single genotype during development did not differ depending on plant genotype sex in weight before (F=2.32, P=0.14, d.f.=1) or after (F=0.02, P=0.89, d.f.=1) pupation. They also did not differ in consumption over 48 h (t=-1.18 , P=0.24, d.f.=1) when fed only one genotype. In the multiple insect- choice experiment, *P. vulgatissima* did not consume more of either male or female plants (t= -0.93, P=0.36).

Pregnant *P. vulgatissima* females oviposited on female *S. viminalis* to a larger extent (285 eggs) than on male plants (238 eggs) in the cage experiment where several beetles were allowed to freely choose between 19 male and 19 female genotypes (z=-2.05, P=0.04 , d.f.=1). The beetles did not, however, consume different leaf areas of either plant sex, and were not more frequently found on clones of either sex.

In the performance experiment, *P. vulgatissima* development time was unaffected by the sex of the *S. viminalis* clone which constituted its feeding plant, both for time until pupation (F=0.22, P=0.64, d.f.=1) and for time until imago (F=0.009, P=0.93, d.f.=1). Pupal weight (F=3.26, P=0.08, d.f.=1) and adult weight (F=0.009, P=0.93, d.f.=1) were also unaffected. Survival during through juvenile instars and pupation also did not differ between beetles that fed on plants of different sexes.

Survival over time for *Anthocoris nemorum* was longer in the treatment where insects were reared on female plant material ($\chi2$=3.88, P=0.05, d.f.=1, Figure 8).

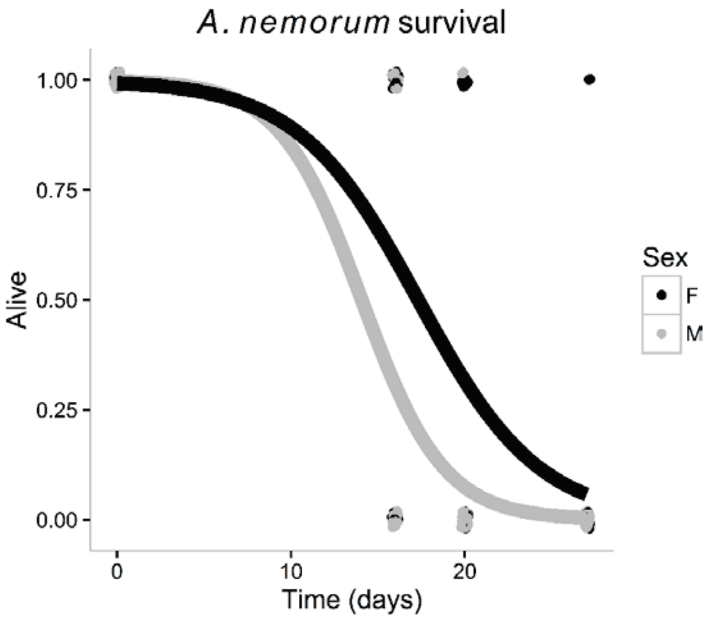

Figure 8. Proportion of surviving predators (*Anthocoris nemorum)* over time in the performance experiment.

Egg predation risk did not differ between the male plant and female plant treatment (t=0.31, P= 0.76, d.f.=1). In addition, plant sex treatment did not affect oviposition during the experiment (F=0.37, P=0.95, d.f.=1), or clutch size

(F=0.00, P=0.97, d.f.=1). Survival time for *A. nemorum* from the start of the experiment was not affected by plant sex (t=0.48, P=0.63, d.f.=1).

4.1.3 Plant chemical analysis

The chemical analysis of the *S. viminalis* leaves revealed 22 plant secondary metabolites (Paper II). My Random forest model included 9 of these and could predict plant sex based on secondary metabolites with an error rate of 24.5% (22.2% for females, 26.7% for males, Figure 9a) among bootstrap iterations, indicating that plant secondary metabolite profiles are informative of plant sex. The compounds included in the final model where primarily derivates of quercetin glycosides, kaempferol-related compounds, and isohamnetin derivates. Female plants had higher concentrations of four of the compounds included (Table 1). All compounds with intersexual differences were selected for the Random forest model.

Table 1. Secondary metabolite concentrations in *Salix viminalis* leaves (mg/g). $\chi 2$ and P-values (bold if significant) are results of effects of sex in linear mixed models (degrees of freedom=1 for each model). Sex is the plant sex with higher concentrations of a compound.

Compound	Female estimates	Male estimates	χ^2	P	Sex
(+)-Catechin	1.20117	0.90069	0.57	0.45	NA
Hyperin	0.13451	0.11151	0.25	0.61	NA
Isorhamnetin 3-acetylglucoside	0.14703	0.15115	0.01	0.94	NA
Isorhamnetin aglycone	**0.03552**	**0.01439**	**5.56**	**0.018**	**Females**
Kaempferol 3-glucoside	0.00154	0.00114	0.66	0.42	NA
Kaempferol 3-rhamnoside	0.06518	0.03078	2.31	0.13	NA
Kaempferol-aglycone + unidentified myricetin derivative	**0.05292**	**0.02240**	**8.38**	**0.004**	**Females**
Monocoumaroyl-astragalin	1.23376	0.91867	0.86	0.36	NA
Myricetin 3-galactoside	0.01531	0.01249	0.79	0.37	NA
Myricetin 3-rhamnoside	**0.02915**	**0.01492**	**3.95**	**0.047**	**Females**
Neolignan	0.04425	0.04607	0.04	0.85	NA
p-OH-cinnamic acid derivative	0.26895	0.24142	0.01	0.91	NA
Protocatechuic acid	0.00031	0.00028	0.30	0.59	NA
Quercetin 3-arabinoside	0.00691	0.00320	0.72	0.40	NA
Quercetin 3-glucuronide	0.00467	0.00315	2.25	0.13	NA
Quercitrin	0.03627	0.01869	2.25	0.11	NA
Unidentified chlorogenic acid derivate	0.02503	0.02227	0.03	0.86	NA
Unidentified lignan 1	0.04000	0.03933	0.01	0.94	NA
Unidentified lignan 2	0.00610	0.00557	0.05	0.82	NA
Unidentified quercetin-derivative	**0.03833**	**0.01533**	**4.57**	**0.032**	**Females**

My Canonical Correspondence Analysis also detected a difference between male and female *S. viminalis* leaf secondary metabolite profiles differed (F= 6.23, P<0.01, d.f.=1, Figure 9b). My calculated standard errors of means for leaf secondary metabolites of *S. viminalis* mixed sex plots, based on measurements of greenhouse plants, were higher for mixed sex plots than monosexual plots (CCA1 0.194, CCA2 0.240 for mixed; 0.117, 0.161 for male and 0.159, 0.160 for female plots).

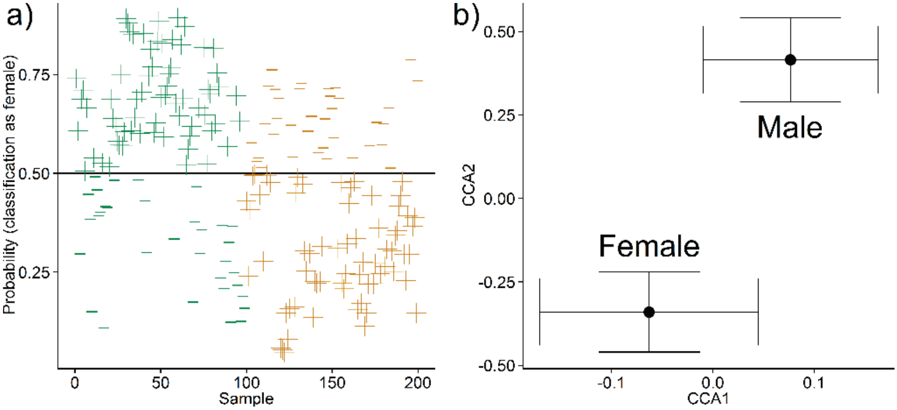

Figure 9. a) Probability of samples to be classified as female plants from re-iterations of a random forest model separating male and female *Salix viminalis* plants based on leaf secondary chemistry profiles. Points above the solid line are replicates classified. Plus symbols represent correct, and minus symbols incorrect, predictions. b) Centroids and standard error bars of male and female plants for the first (CCA1) and second (CCA2) axis coordinates in a canonical correspondence analysis of plant secondary metabolites, clone and sex, explaining 0.37% of total variation.

Total concentrations of phenolic acids ($\chi2$=1.92, P=0.17, d.f.=1, Figure 10) and lignans ($\chi2$=0.29, P= 0.59, d.f.=1, Figure 10) did not differ, indicating that differences between sexes are in profiles, rather than total concentrations, of secondary metabolites. Mean SPAD values, indicating N concentration, did not differ between male and female leaves (t=1.25, P=0.22).

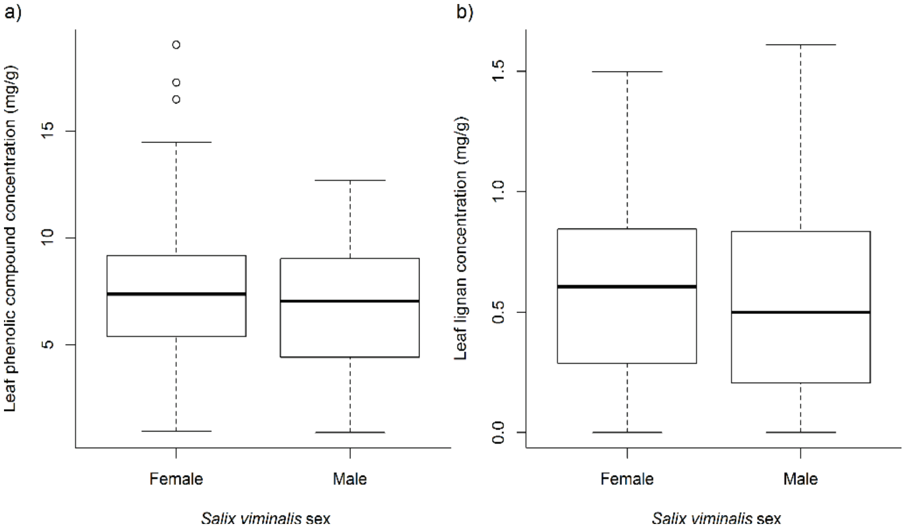

Figure 10. Concentrations of total a) phenolic acid and b) lignan compounds in female and male *Salix viminalis.*

4.1.4 Literature survey of plant sex differences in fungal pathogen infection

I found nine cases (plant-pathogen species pairs) where fungal infection on dioecious plants were female-biased (Paper III, Table 2). Male-biased infections were reported in five cases. In total, I found 13 studies that reported investigations of sex differences in fungal pathogen infection in dioecious plants. Of plant-pathogen species pairs where the tissue that constitute the main infection point was leaf tissue, four cases were female-biased, two male-biased and two unbiased towards either sex.

Table 2. Reported plant sex-biased fungal infections of dioecious plants. *Silene latifolia* was formerly known as *Silene alba* and *Microbotryum violaceum* as *Ustilago violacea*. n.d.= no difference.

Plant	Pathogen	Conditions	Bias	Tissue	Study
Arisaema triphyllum	*Uromyces ari-triphylly*	Field	Female	Leaves	Lovett Doust & Cavers (1982)
Baccharis halimifolia	Unidentified	Field	Male	Leaves	Caño, García-Magro & Herrera (2013); Caño *et al.* (2014)
Buchloe dactyloides	*Puccina kansensis*	Greenhouse	Male	Leaves	Quinn (1991)
Bouteloua dactyloides	*Salmacisia buchloëana*	Laboratory	Female	Flowers	Chandra & Huff (2014)
Cannabis sativa	*Botrytis cinerea*	Laboratory	Female	Flowers	McPartland (1996)
Cannabis sativa	*Dendrophoma marconii*	Field	Male	Leaves	Gikalov (1935)
Juniperus communis	Unidentified	Field	Female	Roots	Ward (2007)
Pistacia vera	*Alternaria alternate*	Laboratory	Female	Leaves	Ash & Lanoiselet (2001)
Populus cathayana	*Melampsora larici-populina*	Laboratory	Female	Leaves	Zhang *et al.* (2009)
Rubus chamaemorus	Unidentified	Field	Male	Leaves	Ågren (1987)
Salix viminalis	*Melampsora spp.*	Field	Female	Leaves	Åhman (1997); Present study
Silene dioica	*Microbotryum violaceum*	Field	n.d.	Flowers	Baker (1947)
Silene dioica	*Microbotryum violaceum*	Field	Male	Flowers	Hassan & MacDonald (1971)
Silene dioica	*Microbotryum violaceum*	Field	Female	Flowers	Lee (1981)
Silene latifolia	*Microbotyrum violaceum*	Field	n.d.	Flowers	Alexander (1989, 1990)
Silene latifolia	*Microbotryum violaceum*	Laboratory, field	Female	Flowers	Alexander & Antonovics (1988); Alexander (1990); Alexander & Maltby (1990); Shykoff, Bucheli & Kaltz (1996); Kaltz & Shykoff (2001)
Silene latifolia	*Microbotryum violaceum*	Field	Male	Flowers	Thrall & Jarosz (1994); Alexander & Antonovics (1995); Biere & Antonovics (1996)

I found seven studies that matched my criteria for my literature survey of sex-biased *Melampsora* spp. infection of *S. viminalis*. Five out of the seven included studies supported female-biased infections being more severe (Table 3). These results should be interpreted with caution however, since most studies included more female than male clones. Because all studies included had used plantations that were mixes of clones and sexes, associational plant sex effects on infection could not be assessed. Four of the studies also used mixed-species plantations (Bell et al. 2001; McCracken & Dawson 2003; Pei et al. 2008; Begley et al. 2009).

Table 3. Studies on severity of *Melampsora* spp. infection on male and female clones of *Salix viminalis*

Study	Settings	Sexual diversity	Assessment	Data	Females/males	Most severely infected
Lascoux et al. (1996)	Lab, inoculation	Mix (growth chambers)	Uredina	Numeric	4/4	Females
Åhman (1997)	Field	Mix	Severity	Ordinal	413/115	Females
Bell et al. (2001)	Field	Mix and monocultures	Severity	Qualitative	3/8	Females
McCracken & Dawson (2003)	Field	Mix and monocultures	Severity	Ordinal	3/7	Males
Pei et al. (2004)	Lab, inoculation	Separated leaves	Severity	Ordinal	23/15	Females
Pei et al. (2008)	Field	Mix	Severity	Ordinal	2/3	Males
Begley et al. (2009)	Field	Mix, clonal monocultures	Severity	Ordinal	3/2	Females
Present study	Field	Mix, sexual monocultures	Severity	Ordinal	20/19	Females

4.1.5 Flower and pollinator experiments

Male plants in clone archives received more pollinator visitations than females (Paper IV) in 2014 (d.f.=1, P<0.001) and in 2015 (d.f.=1, P<0.001). However, there was no difference in visitations to male and female genotypes in the field experiment in 2016 (d.f.=1, P=0.182). Species of hoverflies (Syrpidae) and bees (Apidae) were the most frequent visitors.

Female flowers of *S. viminalis* produced more nectar than male flowers in 2014 (d.f.=1, P=0.003) but not in 2015 (d.f.=1, P=0.495). The number of flowers per catkin was higher in 2015 (d.f.=1, P=0.026). Sugar concentration in floral nectar of female plants was higher in 2015 (d.f.=1, P=0.027) but not in 2014 (d.f.=1, P=0.245).

5 Discussion

This thesis analyses and describes plant sex effects on ecological interactions between *S. viminalis* and consumer organisms. It furthermore compares them to effects in other plants, including closely and distantly related plants, and crops as well as wild plants, in order to broaden our understanding of plant sex effects on consumer organisms. These comparisons underline plant species specificity of ecological interactions, but it also shows how interactions are dependent on the animal or fungal species that the plants interact with. Despite species-specificities of plant sex-biased interactions, common patterns can also be identified.

5.1.1 Plant sex effects on herbivores

I investigated whether *S. viminalis* sex affects abundances and fitness-related life history traits of *P. vulgatissima* consuming male and female plants, in monosexual or mixed-sex fields and in controlled lab environments (Paper I). I only found indications for plant sex effects on *P. vulgatissima* oviposition. A previous study found no plant sex effects of *S. cinerea* sex on oviposition of *P. vulgatissima* (Kabir et al. 2014). There were no effects of *S. viminalis* sex on *P. vulgatissima* development time, body weight, field abundances and survival. Based on resource limitation theory, *P. vulgatissima* would have been expected to have shorter development time, be larger, be more abundant and have higher survival on male plants. The lack such effects was thus in contrast to my expectations based on resource limitation theory and to observations of sex-biased abundances on the closely related *S. cinerea* (Kabir et al. 2014). The differences in effects between the two *Salix* species may indicate plant species-specificity, even among closely related species, of plant sex effects on interactions. For example, leaf morphology or defence related traits may be causing these differences. Another possible explanation for a lack of plant sex effects on *P. vulgatissima* is that generalist herbivores could be more susceptible to variation in plant defences than on specialists such as *P. vulgatissima*. For example, generalist aphids have previously been observed to be affected by *Baccharis salicifolia* sex while a specialist aphid was unaffected (Abdala-Roberts et al. 2016). *Phratora vulgatissima* is specialized on *Salicaceae* species and constitutes and addition to the list of specialists unaffected by plant sex. It is however notable that several other herbivore species, many of which are generalists, have previously been found not to vary in abundances between male and female *S. viminalis* (Åhman 1997). Given the lack of effects on the broad range of investigated insect traits, and the scale of my field experiments, it is unlikely that choosing plant sex, or mixing the sexes will be an effective measure against *P. vulgatissima* herbivory. I instead suggest that research on interclonal variation of other plant traits, perhaps with a more specialized focus on defences, are a promising prospect for producing resistant cultivars.

Roe deer preferred to browse mixed-sex stands (Paper II), which is in line with predictions derived from the detoxification limitation hypothesis. To test whether plant sex or plot sex treatment in the field plots affected roe deer browsing, I measured plant heights as an inverse proxy to browsing. I found that, although there was no difference between male and female plants, plots comprised of a mix of male and female plants experienced more severe browsing. Plant secondary metabolite content may be affecting herbivore preferences through palatability or other sensory perceptions. For instance, there may be a preference towards higher diversity of, over higher amounts of a few, secondary metabolites. Such patterns have previously been found in sheep, where a diet of mixed toxins in low concentrations is preferred over a diet of one toxin at a high concentration (Burrit & Provenza 2000; Villalba et al. 2004). Furthermore, sheep prefer a species-diverse diet with mixed palatability over a monospecific diet with high palatability (Wang et al. 2010). Other examples are of mice reducing toxin intake by mixed feeding (Freeland & Saladin 1989) and the caterpillar *Grammia geneura* feeding more of a diet that contains more diverse toxins (Singer et al. 2002). Secondary metabolite profile is thus likely highly influential for dietary decisions. Because it is common that females of dioecious species are better-defended that males (Cornelissen & Stiling 2005), I also expected total phenolic concentrations to be higher in female *S. viminalis*. However, males and females were similar in total phenolic and lignan concentrations. Although total concentrations did not differ between males and females, they differed in secondary metabolite profile. This indicates that roe deer could reduce intake levels of single compounds by including both male and female plants in their diet. Furthermore, estimations of standard errors of CCA axis 1 and 2 in male, female and mixed sex plots revealed that secondary metabolite diversity in mixed plots were higher. My results thus support the hypothesis that detoxification limitation in generalist herbivores can lead to browsing differentiation within a single plant species that varies in secondary metabolites. This finding may have applicable consequences for management of *S. viminalis* because the plant sex composition of a stand, but not individual plant sex, affects herbivory. Furthermore, other plants with variable secondary metabolite content may be similarly affected by mixing.

Other than secondary metabolite content, nutritional value also plays a role in generalist herbivore choice (Freeland & Janzen 1974). I used chlorophyll measurements as a proxy for N content, which is often limiting herbivores (Moore & Foley 2005) and thus should be of high nutritional value, but found no intersexual differences in *S. viminalis*. I therefore cannot conclude that nutritional value was limiting feeding decisions. Instead, secondary metabolites were likely more decisive. The largest differences between plant sexes were found in quercetin glucoside derivates, a myricetin-kaempferol derivate and an isohamnetin derivate, and it is therefore likely that those were the most important differences in plant secondary metabolite profiles. Quercetin diglucoside, a quercetin glucoside-related compound, is present in higher concentrations in females of *S. myrsinifolia* (Nybakken & Julkunen-Tiitto 2013). There might be differences in plant secondary metabolite content and profiles in plants in a greenhouse environment compared to those in field conditions. Sampling secondary metabolite contents in plants in the field would nonetheless be necessary to confirm this. However, females containing higher concentrations of single phenolics in my experiments do not diverge from general patterns of sex differences in *Salix* spp. and dioecious plants. I also recognise that there are other aspects than N concentration that could be taken into account, such as avoidance of high fibre contents (Drozdz 1979), a diet maximizing soluble sugars (Tixier et al. 1997), or preference of some levels of tannins over none at all (Verheyden-Tixier & Duncan 2000). Further study of these variables in relation to browsing and secondary metabolite profiles could therefore broaden our understanding of generalist herbivore feeding decisions.

Plants survival was lowest in mixed sex plots, which could be expected from browsing patterns under the assumption that herbivory reduces plant longevity. This is further corroborated by heights being correlated to mean survival rates among genotypes. Deer browsing was however not the only antagonistic biotic interaction in the field, but the clear patterns demonstrate that plants in mixed-sex plots have lower survival in field conditions, and this likely is in great part due to herbivore-induced mortality.

5.1.2 Plant sex effects on the omnivorous predator

Crop breeding for indirect defences could be improved by information on effects of traits on biocontrol (Chen, Gols & Benrey 2015; Chen, Gols, Stratton, et al. 2015). In this thesis, I investigated whether biocontrol exerted by *A. nemorum* in *S. viminalis* SRC could be improved by manipulation of plant sex (Paper I). Female flowering *S. viminalis* twigs led to higher survival of *A. nemorum* than male flowering twigs. However, effects of plant sex on *A. nemorum* survival on flowering *S. viminalis* did not translate into increased predation, or on higher field abundances on a plant individual or field level. This pattern is in contrast with earlier findings in the wild relative *S. cinerea*, where development time, assumed to be inversely related to performance, was shorter on male plants and abundances were higher on female plants (Kabir et al. 2014). Because I found no effects on other traits than survival, plant sex likely does not have major ecological implications in the system studied here. Perhaps most importantly, there were no effects of neither plant sex of individual trees, nor of plant sex frequency in a plantation, on abundances. Given the scale and replication numbers of my field experiment, it is unlikely that such effects would have been missed. In an applied context, it is therefore unlikely that *S. viminalis* sex effectively influences biocontrol of *P. vulgatissima* by *A. nemorum*. I did not measure abundances of other predators of *P. vulgatissima* that may be affected by plant traits such as plant sex, and it is possible that there are predators that are affected by plant sex manipulation, however this did not translate into net effects of plant sex on *P. vulgatissima* abundances.

Sex effects on *A. nemorum* survival on *S. viminalis* are in line with the hypothesis that plant trait effects are greater on generalists than on specialists. I found effects on survival only in the experiment where *A. nemorum* were provided with *S. viminalis* only, but not in the tritrophic experiment, where *A. nemorum* could feed on both the plant and on *P. vulgatissima* eggs. This could indicate that the plant food quality is unimportant if prey is abundant. However, the *A. nemorum* used in the tritrophic experiment were not even-aged at the start of the experiment, which could have greatly increased variation in survival time in the experiment. Differences in results between experiments may also be a result of dependence on seasonality, since the bitrophic experiment was performed on flowering twigs in spring and the second experiment on vegetative shoots during late summer.

5.1.3 Plant sex effects on fungal pathogens

My study demonstrated *S. viminalis* sex effects on *Melampsora* spp. infections of single plants, where female plants were more severely infected (Paper III), which is in line with previous findings for this plant (Åhman 1997). However, I found no effects from plot sex treatment, indicating that plant sex only have direct effects on *Melampsora* spp. infection severity and that no associational plant sex effects are present, at least in this system. My literature survey on *S. viminalis* infected by *Melampsora* spp. further supports my field results that females are more severely affected. My literature survey on fungal pathogen infections in dioecious plants further supported the finding of female plants of dioecious species more often being more susceptible or severely affected than male plants.

I found no evidence for associational effects of plant sex of surrounding plant individuals on focal plants, regardless of sex and genotype of the focal genotype. This does not support my hypothesis that a mix of plants of different sex would reduce infection, and contrasts findings that other sources of genetic diversity reduce fungal pathogen infection (e.g. Zhu et al. 2000). One difference between my experimental design and most SRC plantations is that my plantations held higher genotypic variation, with 20 genotypes per plot being used over the field experiment. Because the severity of fungal pathogen infection have been reported to be lower in clone mixes (Begley et al. 2009), the high diversity may therefore have decreased the infection at my field sites compared to what monoclonal plantations, or even low-diversity plantations, would have experienced. I could not attribute these sex effects to specific traits such as intersexual differences in leaf size.

Resource allocation theory predicts that female plants are better-defended and therefore suffer less severe pathogen infections than males (Vega-Frutis et al. 2013). On the contrary, female plants were more severely affected by *Melampsora* spp. infection. In addition, my literature surveys suggested that this pattern is most common not only for *S. viminalis* and *Melampsora* spp., but for dioecious plants in general. For 12 plant species, I found nine studies supporting female-biased infection, and six supporting male-biased infection, being prominent. The results from my field study in combination with the literature surveys strongly suggest that resource allocation patterns in relation to reproduction is not likely the most important mechanism underlying plant sex-biased fungal pathogen infections in dioecious plants.

Potential sources for differences in susceptibility between male and female dioecious plants to pathogens include a variety of plant or pathogen traits. Differences in exposure (size or exposure time), secondary metabolite content or mechanical defences have all been suggested as plant traits that may affect interactions with antagonists differently depending on plant sex (Ågren et al. 1999), and these types of traits can all differ between plant sexes. For example, phenology can differ between the sexes (Conn 1981), leaf areas are larger in females of some *Salix* spp. (Ueno & Seiwa 2003), female plants may contain larger amounts of phenolic compounds (Nybakken & Julkunen-Tiitto 2013) and mechanically damaged female plants produce leaves with more trichomes in *Urtica dioica* (Mutikainen et al. 1994). In my field- or lab experiments, I did not find support for any specific mechanism underlying plant-sex biased infection severity. I found no intersexual difference in leaf area or phenology, suggesting that exposure is not a main driver of sex differences. Furthermore, total phenolic compounds were not different between male and female plants. Secondary metabolite profile differences, however, suggest that the specific compounds contained within leaves could have led to females being more severely infected. Further studies investigating manipulating levels of secondary chemistry and including infected leaves among chemical samples would be needed in order for the mechanism of secondary chemistry on plant sex-biased infection to be evaluated. However, previous studies have not found relationships between secondary metabolite content and *Melampsora* spp. infection in *Salix myrsinifolia*. As for mechanical defences, *S. viminalis* leaves only have short trichomes. I did not measure trichome density but it is possible that any differences in density could affect infection. Further research is needed on the mechanisms underlying plant sex-biased differences.

5.1.4 Plant sex effects on pollinators

My results revealed that male *S. viminalis* receive more frequent visitations by pollinators (Paper IV). This was expected based on the fact that pollen is a valuable resource for bees in spring (Reddersen 2001) and based on observations in other dioecious species (e.g. Elmqvist et al. 1988; Vaughton & Ramsey 1998). Higher visitation rates to males may also have been a result of more flowers per catkin. Female floral nectar, however, in one of the years contained more sugar than male nectar and in the other year female flowers produced more nectar. Females producing nectar containing more sugar has been previously observed in other plant species (Ashman 2000; O'Rourke et al. 2014) and could be a mechanism of compensating for not producing pollen as a reward. However, because males were more visited in two rounds of observation out of three, they are likely the sex preferred by pollinators, and thus most likely the most rewarding.

Strawberry fruit production was higher in mixed-sex *S. viminalis* plots in 2015. The increased yield in the mixed-sex treatment indicates that pollination, as a legacy effect of male and female plants in that treatment, was likely higher. In 2015, flowering time of *S. viminalis* had an overlap with that of *F. vesca*, while in 2016 there was no overlap of flowering times. In a previous study, 78% of individually marked bumblebees can continue to visit a site after the flowers of their target plant species have been removed and, by switching to flowers of another plant species with overlapping flowering time (Ogilvie & Thomson 2016). The interannual difference in overlapping phenology between the two species in my experiments may explain why there was a legacy effect on strawberries in 2015 but not in 2016. Indeed, one may expect that legacy effects decrease with less overlap and longer times between flowering times. My study nonetheless indicates that legacy effects may be used as a means of increasing pollination services. This result may therefore be applicable in agricultural settings, where *Salix* SRC plantations can be manipulated to include a mix of male and female plants to increase yields in nearby pollen-limited crops, possibly through legacy effects.

5.1.5 Summary of discussion

This thesis constitutes a broad investigation on plant sex effects on antagonists. I have shown here that plant sex of a dioecious species can have very different types of effects on consuming organisms. These effects may for example depend on generality of the herbivore, the organism groups or timing of the interaction. However, I also found general patterns: *S. viminalis* and many other dioecious plants experience female-biased fungal infections. Stand- or population effects of plant sex composition on consumer interactions, especially on interactions with herbivores that may readily move and make foraging decisions on larger scales, should be further investigated. I also found that plant sex effects on insects found in laboratory experiments did not lead to differences in field abundances of the insects. Finally, from an applied perspective, effects of plant sex on pollinator communities and legacy effects as well as spill-over effects of plant sex on pollinator visitation are promising and should be further investigated.

6 Conclusions

There are common patterns for plant sex effects on plant-consumer interactions, but they can also be species-specific or depend on the type of interaction. Based on my results, my recommendations for management of *S. viminalis* is to utilize male clones for improved resistance against the most severe fungal diseases, *Melampsora* spp., and for potentially beneficial effects on nearby pollinators and pollination-related ecosystem services. I do not recommend mixing the male clones with female clones in unfenced plantations because of risks of higher herbivory from roe deer. Note however, that mixing the sexes may have positive effects on nearby pollen-limited crops and other plants. The deviations from the expected and general patterns of male-biased herbivory that my study of *S. viminalis* interacting with several consumer organisms indicate that more research is needed for a better understanding of the mechanisms underlying plant sex-biased consumption.

6.1 Plant sex and biotic interactions

Male-biased herbivory is more common than female-biased herbivory (Cornelissen & Stiling 2005), but results from my experiments did not confirm to this general pattern. I found limited or no direct effects of plant sex on herbivorous insects, or on omnivorous insects. Roe deer however were affected by mixing plant sexes but did not browse one plant sex more than the other. I conclude that plant sex ratio effects can affect herbivory but I cannot draw conclusions about what caused roe deer, but not *P. vulgatissima*, to be affected. One possibility is that specialists and generalists differ in how they are affected by biochemical defences, and another is that the scale at which herbivores make foraging decisions affect their selectivity.

Fungal pathogen infection in dioecious plants are primarily female-biased and, at least in *S. viminalis*, the effects of plant sex act at a plant individual scale. I have demonstrated that plant sex composition of neighbouring *S. viminalis* stands do not modulate the strength of individual plant sex effects, which indicates that mixing male and female plants does not affect infection. The mechanisms leading to female-biased infections in *S. viminalis* in particular, and plant sex-biased infection dioecious in general, is in need of investigation if I are to understand plant-sex biased infections and be able to predict effects on pathogens.

Because pollinators seemed to prefer visiting male plants over female plants, I conclude that male plants likely are inherently advantageous as food sources, probably because of pollen offered to visitors. However, my results also indicate that visitation may be increased by mixing male and female plants, and using mixes for promoting pollinator communities should be investigated as a means of increasing production of pollinated crops and pollinator populations.

6.2 Impact on *Salix viminalis* Short Rotation Coppice

I conclude that genotypic effects exist in *Salix viminalis* SRC on biotic interactions and that genotypic variation likely has a major impact on ecological interactions in plantations. It has previously been proposed that clone-specific effects on interactions are larger than plant sex effects and that plant sex therefore do not need to be considered (Åhman 1997). In the light of my study, I can say that this is true for most interactions with insects, but I can also see that there might be an impact on mammalian generalist herbivores and on fungal pathogens.

I have demonstrated that male *S. viminalis* are preferred over females by pollinators, and that females are more susceptible to fungal disease. I therefore recommend that male plants are planted in SRCs, especially if pollen-dependent crops are adjacent to plantations. Whether the male clones should be planted together with female clones depends on a balance between the potentially increased pollinator-related ecosystem services and the raised risk of herbivory by roe deer.

7 Future perspectives

This thesis presents results underlining the importance of plant sex for plant-consumer interactions. However, I also concluded that i) plant sex effects on plant-consumer interactions can be species-pair specific, and ii) scale is important for trait-mediated effects on interactions. Species-pair specificity may not sound promising to researchers wanting to develop general frameworks for understanding and predicting interactions. It is however clear that we need to understand which traits in plant and consumer species affect interactions and I therefore propose using a broad-scale trait based focus. Scaling up our understanding from a plant individual perspective to groups and populations, from annual effects to periods of times spanning several years, and from local effects to landscape-scale effects, require large scale experiments, both in time and space. It is promising that many of the trait-based trophic interactions are already well-studied. Testing the scalability of current knowledge will provide new opportunities for development of interaction-based ecology and its applications.

8 References

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