Sex effects of dioecious plants on interactions with insects: considering herbivores, pollinators and effects on a third trophic level

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Introductory Research Essay
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Introductory essay

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Introduction

Aims of this essay

Bioenergy forestry is a viable alternative to fossil fuels, providing a mean to reduce net carbon emissions and thus our impact on climate change. It is therefore important that bioenergy production is not hampered by herbivory. If plants with certain traits can be chosen to maximize biological control, bioenergy forestry can become an even more potent and sustainable practice. The aim of my project is to investigate what effects sex in a bioenergy crop has on multitrophic interactions and pollinator attraction. Plant sex effects will be investigated using the dioecious *Salix viminalis* (Salicaceae), commonly grown in plantations in Sweden, Germany, Denmark, Great Britain and a few more European countries. The project will describe how plant sex affects ecosystem services provided by this crop (biological control and pollination), and through that the potential to increase these ecosystem services through selecting or combining clones of either sex. While the results will be easily applied knowledge, my project will also expand our general knowledge of plant sex effects; multitrophic interactions between plants and insects and among insects are barely described. Insect species under study will be a herbivorous beetle (*Phratora vulgatissima*, Coleoptera), an omnivorous predatory bug (*Anthocoris nemorum*, Hemiptera), and a parasitoid wasp (*Perilitus brevicollis*, Hymenoptera). I will use these species in a series of field- and laboratory based experiments. In addition, plant sex effects on pollinator attraction will be tested in the field experiments through observing flower visitation rates and recording berry production in adjacent woodland strawberries, *Fragaria vesca*. The aim of this essay is to compile (i) an overview of previous work performed on, or otherwise relevant to, plant sex effects on interactions with animals and (ii) necessary information on the study system that I will use in the planned experiments.

Knowledge gaps and hypotheses

So far only a handful published experimental studies have investigated plant sex effects on multitrophic systems (Mooney et al. 2012; Mooney et al. 2012; Petry et al. 2013). This is somewhat surprising since a large proportion of the described plant species, approx. 10%
(Geber 1999), are dioecious and all species are involved in multitrophic interactions. The substantial body of literature that treats other genetic plant effects on trophic interactions contains evidence for effects on insect densities (e.g. Fritz & Price 1988), herbivory (e.g. Stiling et al. 1996), ovipositioning rate (e.g. Lehrman et al. 2012), parasitism (e.g. Rand et al. 2012) and preference (e.g. Cronin & Abrahamson 1999) of insects of different trophic levels. Studies of plant sex effects on such traits and interactions in applied systems including dioecious plants would allow for evaluation of effects on valuable ecosystem services, such as indirect defence through biological control efficiency and pollination. In the part of the project that concerns biological control I take an approach where plants, herbivores and predators are considered, with a study system encompassing three trophic levels. I will also look at plant sex effects on pollinator visitation, but this essay will focus on tritrophic interactions and biological control. The only published study of sex-biased herbivory in *S. viminalis* includes gall midges and lepidopterans (Åhman 1997). There are no studies of plant sex effects on the blue willow leaf beetle, the insect herbivores causing the most severe damage on *Salix* Short Rotation Coppice (SRC) (Björkman & Liman 2005). An investigation of sex effects on the multitrophic interactions would assess the potential of increased biocontrol by choosing plants of either sex, a simple and inexpensive solution to a great challenge. I therefore want to investigate *S. viminalis* sex effects on herbivores and their natural enemies, as well as on pollinators. The general questions I will address are whether plant sex affects: 1. the plant’s interactions with herbivores; 2. the biological control efficiency exerted by natural enemies of the herbivores; 3. pollinator visitation rate on *S. viminalis*, species composition of visiting pollinators, and pollination of.

**The project**

To assess the above stated hypotheses I will perform several experiments in field conditions. I will use 30 plots with female, male or both male and female plants to compare plant sex effects on abundances of a herbivore and two types of biological control agents; omnivores and parasitoids (Fig. 1.). To better understand the mechanisms that lead to differences in abundances, I will also perform experiments where I test whether (i) plant food quality for the insects is plant sex dependent, (ii) the insects use olfactory cues to navigate to plants of either sex and (iii) the omnivore’s predation efficiency is affected by host plant sex. To test for plant sex effects on pollinator attraction I will also study pollinator visitation in terms of visitation frequency and pollinator diversity at *S. viminalis* and neighbouring woodland strawberries (*F. vesca*) in fields with *S. viminalis*.

All species being common in the study area, all experiments posing low or no risks of negatively affecting nearby ecosystems and the project being funded by a governmental department, there are to my knowledge no ethical issues connected to my project.
Figure 1. An overview of the study system showing interactions that will be examined between the plant (bottom), the herbivore (top mid), the parasitoid (right) and an omnivorous predator of the herbivore (left). Interspecies interactions are referred to in as (a - e) throughout the essay.

**Theoretic background**

**Plant sex effects on interactions**

The plant traits that mediate effects on species of different trophic levels have to be identified in order for insights to be applicable or used in a more generalised context (e.g. for biological control programmes and understanding of other study systems). Plant traits can affect prey or predators directly (Fig. 1. a-c), or indirectly one through the other (Fig. 1. d-e), and may have feedback effects onto the plant (Mooney & Singer 2012) through the herbivore (Fig. 1. a).

Below I discuss both direct and indirect plant-induced effects on interactions between trophic levels and discuss effects of plant sex that I will investigate in my project.

**Sex-biased herbivory**

There is a large body of literature on sex-biased herbivory (Fig. 1. a) on dioecious plants, reviewed by Cornelissen & Stiling (2005), Ågren et al. (1999) and Boecklen et al. (1993) with male-biased herbivory being the common finding of most studies (e.g. Boecklen 1990; Capeda-Cornejo & Dirzo 2010; Elmqvist & Gardfjell 1988, Alliende & Harper 1989, but
see Åhman 1997; Mooney et al. 2012). There are also many studies of herbivore performance on dioecious plants (e.g. Lehrman et al. 2012; Peacock et al. 2004) in which differences in herbivory between single genotypes were found. Some of these differences are likely affected by plant sex. Differences in resistance to herbivores may be caused by differences in the balance of the trade-off between relative fitness cost of lost biomass and defences. Sex-biased herbivory, often towards males, may be a result of differences in trade-offs between sinks of resource allocation within a plant. An often cited explanatory hypothesis is that male plants allocate more resources to vegetative growth and less to defences than female plants, and thus compensate for lost biomass, while female plants defend themselves better at a cost of growth rate (e.g. Ågren et al. 1999; Cornelissen & Stiling 2005). This is because female plants can have a higher reproductive effort (Lloyd & Webb 1977) that lasts for a longer time period. It could thus be that herbivory is a driver in the evolution of dioecy (Cornelissen & Stiling 2005).

The preference-performance hypothesis

The preference-performance hypothesis, predicting that a herbivore’s host preference should agree with its, or with its offspring’s, best performance, has received support in a recent meta-analysis (Gripenberg et al. 2010). For a better understanding of ecological relationships, and the mechanisms driving them, studies on insect performance alone can be completed with those on preference. In my project, I expect to see that preference and performance (survival, development and reproduction) concur for both the herbivore (P. vulgatissima) and for the omnivorous predator (A. nemorum) on S. viminalis.

Female insects can be expected to make a better choice than males (Gripenberg et al. 2010) because ovipositioning decisions strongly affect fitness. Females can especially be expected to make a better choice if the optimal diet differs between insect life stages and the fitness advantage of selecting a suitable plant for eggs and larvae supersedes the fitness loss of foraging on a food source suboptimal for adult insects. Of course, male insects should also be attracted by females, and likely prefer the same plants, but the selectiveness may nonetheless be higher for female insects.

Tritrophic interactions and biological control

Our understanding of plant sex effects on higher trophic interactions (Fig. 1. d-e) is in need of development. While a large body of studies describes the effects of plant sex on herbivores and herbivory (reviewed by Ågren et al. 1999 and Cornelissen & Stiling 2005), less is known about effects on species of higher trophic levels such as predators and parasitoids (Fig. 1. b-c). One study (Mooney, Fremgen, et al. 2012) found differences in abundances of natural enemies and aphids between herbivore-induced male and female Valeriana edulis. They did however not detect differences between uninduced male and female plants, and Petry et al. (2013) found only a marginally significant difference between male and female plants when they estimated plant sex effects on abundance of all predators of aphids in the same uninduced study system. Since herbivores and omnivorous predators may have different
optimal plant food sources (Stenberg et al. 2010; Stenberg et al. 2011), investigating whether predator and herbivore performances are differently affected by sex of dioecious plants is important for understanding the function of the predator as biological control agents. If a herbivore and its predator both perform better on plants of one sex, or if the predator performs equally well on both sexes, the biological control efficiency of the predator may be unaffected by sex of the host plant. However, if the herbivore and its predator perform better on plants of different sex, that difference may influence the effectiveness of biological control agents in single sex commercial plantations. Effects of plant genotype can thus also have indirect effects on the plant itself through interactions with herbivores and predators. One perspective of viewing the way that a plant can affect (I) the herbivore (Fig. 1. a) and (II) the herbivore’s predators (Fig. 1. b-c) is provided by dividing effects into (a) effects on density and (b) effects on traits (Mooney & Singer 2012). The most relevant traits are consumption rates, reproduction and longevity. Effects that increase herbivore densities can be expected to increase the densities of its predators indirectly through the herbivore density, while higher predator density suppresses herbivore density and feed back to the plant as lowered herbivory. Traits can be expected to similarly affect the interaction either directly or indirectly.

A predator’s response to plant sex (Fig. 1. b-c) may depend on its diet breadth, which in that case affects the potential as a biological control agent. Numerous studies have tested the efficiency and effectiveness of both generalist (e.g. omnivorous) and specialist (e.g. parasitoid) predators, and Symondson et al. (2002) has compiled their advantages and disadvantages. Stiling & Cornelissen (2005) found in their meta-analysis of biological control agent traits that although specialists have often been held forth as superior biological control agents, generalist predators have larger effects on pest abundances than specialists, and suggested that this may be due to generalists’ ability to switching target prey. Another paper specifically reviewed generalist predators as biological control agents, and concluded that about 75% of experimental studies found a significant suppressive effect on abundance of targeted species (Symondson et al. 2002). Omnivorous species can be advantageous as biological control agents in that the population may persist during periods of low abundance of the intended prey (Ågren et al. 2012; Lehrman et al. 2013). If a generalist predator alters its feeding behaviour towards the plant, the predator’s consumption of plant tissue might risk overweighing the positive effects (Stenberg et al. 2011; Lehrman et al. 2013), but if the predators preference reflects its performance, the predator’s population size may nevertheless decrease with its prey. Whether a generalist predator, especially an omnivorous predator, reaches its potential as a biological control agent should depend on plant quality because the plant may be an even more important as a food source for periods of low prey density. Meanwhile, specialists may have higher predation rates in times of high prey abundances and may depend on high plant quality during a short time of their life cycles (e.g. nectar foraging during nymphal stages). If plant sex determines nutritional quality for predators, it is important to investigate whether generalists and specialists are differently affected.

A plant’s maintainability of the predatory (Fig. 1. b) or parasitic (Fig. 1. c) insect’s populations is partly determining the insects’ suitability as biological control agents. The
maintainability is affected by whether it is a part of their life-cycle to stay within the intended area of biological control, not least between growing seasons, and together with their prey/host. A predator that overwinters on or close to the plants may be advantageous for biological control purposes because of interannual population stability. The population is however then likely to collapse at a harvest (see Björkman et al. (2004)) or by other strong disturbances in the system. Parasites or parasitoids may on the other hand be naturally re-introduced as it follows the herbivore to the host plants. Predators that spend part of their life-cycles away from both the herbivore and the plants may be a challenge to maintain in population sizes effective for biological control. Since the dependence on plant food quality may differ between these types of biological control agents, any differences in their preference and performance on male and female plants is likely to affect their performance based on their type of life-cycle or diet. Differences in diet requirements (e.g. dependence of seasonal dependence on sap or floral nectar) can therefore also affect maintainability of a biological control agent’s population, depending on plant sex.

Plant quality may indirectly influence parasitism (Fig. 1. e). Bukovinszky et al. 2009 found that the parasitoid Cotesia glomerata had lower parasitism success if the host caterpillar Pieris rapae were feeding on herbivore-induced cabbage (Brassica oleracea) than when P. rapae were feeding on uninduced plants. They also found a difference between parasitism success of C. glomerata on P. rapae feeding on cultivated and wild plants. Both differences found were at least partly caused through prey size, which likely indicates the pathways for the parasitoids dependence on plant quality. If herbivores prefer plants of either sex it is thus likely that the herbivores found on plants of their preferred sex are preferred by parasitoids. The finding that pre-induced plants lead to lower parasitism rates of herbivores have also been found by other authors (Fellowes et al. 1998), indicating that parasitoid efficiency may be lowered by induced defence substances if outbreak levels are low. Another aspect speaking against parasitoids as biological control agents is negative density dependence through parasitism that fails due to attempts on already parasitized hosts.

Simultaneously utilising several species of biological control agents (Fig. 1. b–c) have been reviewed to enhance the suppressive effect on pest abundance by on average 27.7% compared to using a single biological control agent (Stiling & Cornelissen 2005). That review did however neither consider taxonomy nor predatory guilds. One study has found a multitrophic interaction where the addition of generalist predators decreased the parasitism rate by a parasitoid wasp on aphids (Snyder & Ives 2001), possibly by mainly foraging upon infected, and thus weakened, prey. If energy conversion between prey and parasitoid is efficient, and the parasitoid and predator act on the same life stages, infected prey might perhaps even be of higher nutritional quality for predators through indirectly varying its nutrient content. More studies of predator effects on parasitism rates in pests could contribute to the understanding of what effects the combination of biological control agents has, and whether plants have different genetic (e.g. plant sex-dependent) effects on generalists and specialists performance.
Study systems and project description

To investigate the main hypotheses of this project, a system with species of different trophic levels and guilds are studied; a dioecious plant, an insect herbivore, pollinator insects, an omnivorous insect predator, and an insect parasitoid (Table 1.).

Table 1. Hypothesized effects on effects of S. viminalis sex on interactions with insects.

<table>
<thead>
<tr>
<th>Interaction agent</th>
<th>Sex effects on interaction agent</th>
<th>Effect on plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbivore (<em>P. vulgatissima</em>) (Fig. 1. a)</td>
<td>Population size, herbivory rate</td>
<td>Herbivory rate (directly); growth (directly) (Fig. 1. a)</td>
</tr>
<tr>
<td>Predator (<em>A. nemorum</em>) (Fig. 1. b)</td>
<td>Population size, predation efficiency</td>
<td>Herbivory (indirectly) (Fig. 1. d)</td>
</tr>
<tr>
<td>Parasitoid (<em>P. brevicollis</em>) (Fig. 1. c)</td>
<td>Parasitism rate</td>
<td>Herbivory (indirectly) (Fig. 1. e)</td>
</tr>
<tr>
<td>Pollinators (Bumblebees, bees and hoverflies)</td>
<td>Population size, visitation rate, visiting species</td>
<td>None, or on resource allocation</td>
</tr>
</tbody>
</table>

*Salix viminalis* is a dioecious willow species. The main usage of *S. viminalis* is renewable production of energy in SRC, making maintenance of biomass production an important challenge (Torp et al. 2013; Lehrman et al. 2012). Most of the plantations are large monocultures (Ramstedt 1999; Dalin et al. 2009), which has been criticised for increasing risks of disease selection and spread (Ramstedt 1999), and increased risk of pest insect outbreaks (Dalin et al. 2009). The plant biodiversity within plantations is very low, although some positive effects from plantations on diversity of birds (Berg 2002) and arthropods on a landscape level (Rowe et al. 2011) have been found. Many of these monocultures are monoclonal and thus monosexual (Reddersen 2001). Whatever effects *S. viminalis* sex may have on trophic interactions, they can therefore be actively influenced by choice of clones of either sex or of both sexes.

It has been noted that dioecious plants are well suited for studies of resource trade-offs (Capeda-Cornejo & Dirzo 2010) between costly sinks. One common resource sink is secondary substances, and part of the project will address questions of plant sex and their direct defence. Since clones of a certain sex can be chosen when planted in a field, it is of interest whether either sex have different effects on herbivory. Although *S. viminalis* is a fairly well-studied system, the literature mostly describes differences between single clones or female clones, currently limiting the *a posteriori* generalisations that one can make about
differences between clones of different sex. However, secondary metabolites often play a major role in plant defence against herbivores and one study of another Salix species, S. myrsinifolia, have found sex-related differences in concentrations of phenolic compounds (Ruuhola et al. 2001), so it is likely that some differences are present in S. viminalis too. Additionally, information on how herbivores and their enemies react on volatiles emitted by male and female plants would provide insight into function of indirect defences.

Some work has already been performed on herbivory and traits affecting herbivory in Salix systems. One previous study did not find differences in presence of attacks by gall midges and lepidopterans between plants of different sex of S. viminalis in a field experiment (Åhman 1997), and that is currently the only study of insect herbivory and S. viminalis sex. My project will mainly focus on a coleopteran herbivore, P. vulgatissima, and some of its natural enemies. Damage by mammal herbivores will also be recorded, the most likely mammal herbivores being the European roe deer (Capreolus capreolus) and the European hare (Lepus europaeus). There are a couple of studies investigating relationships between plant sex and herbivory from mammals in other Salix species. Mountain hares (Lepus timidus) have been identified to prefer male twigs of S. caprea and S. pentandra (Hjältén 1992) and one study found a preference by voles (Microtus agrestis) for bark of male S. myrsinifolia (Danell et al. 1985), while another study found no difference in lemming herbivory on S. lanata (Predavec & Danell 2013).

Other effects of the sexual dimorphism of plants may origin from the flowers. Pollen can be collected along with the nectar for Salix-visiting species (Kevan & Lack 1985), and in S. viminalis, male catkins produce both nectar and pollen. The fate of the catkins after the species’ peak flowering period differ; male catkins wither and fall off after flowering while female catkins develop (Reddersen 2001), and that could cause a difference in nectar availability in either direction. Additionally, Reddersen (2001) noted a tendency of female S. viminalis to produce more flowers than male plants did. Such differences in nutrient content and temporal availability, and any differences in scent, of flowers might affect pollinators and shape the fauna of natural enemies of herbivores. I will therefore investigate effects of plant sex on a number of species that may act as herbivores, biological control agents, or as pollinators. In my experiments, twenty haphazardly selected commercial Swedish clones of each sex will be used.

Phratora vulgatissima (Coleoptera: Chrysomelidae) is a herbivorous leaf beetle commonly found on different Salix species. Salix viminalis is one of the more susceptible species (Stenberg 2012; Torp et al. 2013), and P. vulgatissima is the most serious pest on Salix short-rotation coppice (Peacock, Lewis & Herrick 2001; Peacock, Lewis & Powers 2001) and the risk for outbreaks of P. vulgatissima is higher in monocultures than in natural conditions (Dalín et al. 2009), which above all makes it problematic for agriculture. The adult beetles lay eggs from late May to late June (Björkman et al. 2000), and the hatched larvae cause the majority of all defoliation (Lehrman et al. 2012) during their development until pupation. The pupal stage is spent in the soil after the third instar (Björkman et al. 2000; Torp et al. 2013). Differences in susceptibility of Salix spp. to P. vulgatissima has been attributed to feeding
preferences (Peacock, Lewis & Powers 2001). Feeding preferences are likely to be dependent on defence substances produced by the plants as a direct defence. Studies of phenolic substances, which often are associated with defence, have found differences in concentrations between clones of *S. viminalis* and other species of *Salix* (Lehrman et al. 2012; Torp et al. 2013). As mentioned in the theory section, females can be expected to make a better choice for their total fitness. Furthermore, their ovipositioning will determine where the larvae will consume leaf biomass I will therefore keep track of *P. vulgatissima* sex during my experiments.

*Anthocoris nemorum* (Heteroptera: Anthocoridae) is an omnivorous insect that when present in *S. viminalis*-plantations forage on *P. vulgatissima* eggs and larvae, other small animals and plant material. It overwinters in the *Salix* stands (Sage et al. 1999) in SRC, and thus the population decreases after a harvest, interrupting the biological control effect it exerts afterwards (Björkman et al. 2004). However, it has strong advantages. *Anthocoris nemorum* populations persist between non-harvest years and has been demonstrated to be capable of survival and development on prey-free *S. viminalis* plants (Stenberg et al. 2010), where they primarily feed on plant sap. Although many studies have investigated plant sex effects on herbivores there is a lack of data on plant sex effects on sap feeding herbivores (Mooney, Pratt, et al. 2012). Interestingly, *A. nemorum* has been found to be behaviourally affected by *Salix* spp. clones: They are demonstrated to have a higher chance of survival until adulthood when reared on a clone resistant to herbivory exerted by *P. vulgatissima* than when reared on a susceptible clone, corresponding to *A. nemorum*’s preference when placing potted plants randomly in the field (Stenberg et al. 2010). *Anthocoris nemorum* is also able to discriminate between different *Salix* sp. by their volatiles and have preferences amongst these that alter depending on herbivore damage (Lehrman et al. 2013). The concurrence of preference and performance is yet to be tested for more genotypes in order to draw broader conclusions, such as whether plant sex affects the preference, preference-performance relationship and how that relates to herbivore presence. I therefore want to test experimentally whether the preference-performance conformity is consistent on clones of both sexes.

*Perilitus brevicollis* (Hymenoptera: Braconidae) is a parasitoid wasp. As an adult, it consumes the nectar of *Salix* spp. It utilizes *P. vulgatissima* as a vessel for parasitoid larval development, after which the leaf beetle dies. Thus it has potential as a biological control agent. As *P. brevicollis* overwinters as a larva in *P. vulgatissima*, they do not stay by the host plants the whole year, but follows *P. vulgatissima* to their overwintering sites and subsequent host plants. The rate of *P. vulgatissima* interannual returns to *S. viminalis* fields is presently unknown, but can be expected to depend on surrounding habitats, local population size and *S. viminalis* stand age. Over a longer time span, the parasitoid should therefore have population dynamics and potential for sustenance closely coupled to *P. vulgatissima* behaviour. As the life cycles differ between *P. brevicollis* and *A. nemorum*, I want to detect whether this systems behaviour conforms to the idea of plant sex affecting biological control agent life cycles differently, as discussed in the theory section. If omnivore populations are negatively affecting parasitoid populations, or if plant sex have different effects on these, I also expect to find a negative correlation between the *P. brevicollis* and *A. nemorum* densities.
The main pollinators of Salix sp. are bumblebees (Bombus spp.), but Salix spp. also constitute one of the most attractive nectar sources for hoverflies (Diptera: Syrphidae) (Branquart & Hemptinne 2000). Salix spp. are important food sources for pollinators early in the season, and may therefore affect pollination success in adjacent wild plants and crops that depend on insects for pollen transfer. Any differences between sexes (or clones) in Salix viminalis with respect to nectar quality, or (and) effects of its pollen as a food source, may therefore affect the ecosystem services provided by bumblebees and hoverflies.

Summary

Little is today known of plant sex effects on higher trophic interactions. A majority of the studies investigating differences in herbivory on plants of different sex in diocious species have found a bias in herbivore abundance or the extent of herbivory, most commonly toward male plants. While many studies have investigated plant sex effects on single interacting animal species, mostly herbivores, only a handful of publications discuss similar effects on multispecies- or multitrophic interactions. Furthermore, most of the knowledge of plant sex effects are on direct effects while much less is known about indirect effects and feedback effects of plant sex within multitrophic systems. Numerous studies have assessed the potential of enemies of pest species as biological control agents in other contexts. I take a plant-centred approach and focus on plant sex effects on two indispensable ecosystem services; biological control and pollination. My project will describe the plant sex effects on species of different trophic levels through experiments comparing traits and densities. I will do that using a system with commercial S. viminalis clones commonly used to produce bioenergy, and its most important interacting insect species in field- and laboratory based experiments. My ambition with this project is to expand our general knowledge of plant genetic effects on multitrophic interactions, and while doing so increase the potential of Salix SRC to mitigate our impact on the changing climate.

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