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# Plant sex effects on insect herbivores and biological control in a Short Rotation Coppice willow

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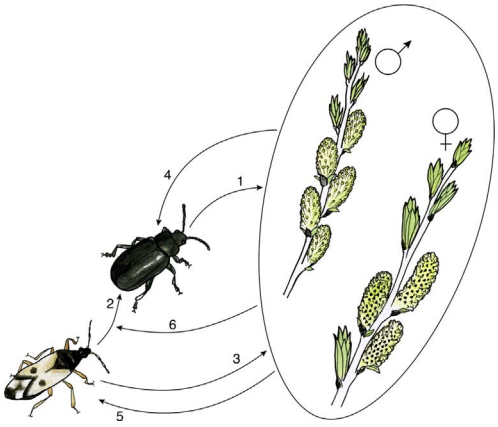
## GRAPHICAL ABSTRACT

Tritrophic interactions that are investigated in the studied system: 1. Herbivory by the herbivore (*Phratora vulgatissima*), 2. Predation by the omnivore (*Anthocoris nemorum*) 3. Herbivory by the omnivore, 4. Plant (*Salix viminalis*) sex effects on the herbivore and its herbivory, 5. Plant sex effects on the omnivore and its herbivory, 6. Plant sex effects on predation.

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## ABSTRACT

In the wild, plant sex can affect plant-herbivore interactions and higher trophic levels, including natural enemies of the herbivores. However, the possibility of manipulating plant sex to improve biological control and reduce herbivory in domesticated dioecious crops remains unexplored. The dioecious bioenergy crop, *Salix viminalis*, is often planted in monoclonal, and thus monosexual, fields. We investigated whether using plant clones of either sex, or mixing plants of both sexes, reduced the performance and abundance of the herbivorous pest insect *Phratora vulgatissima* and its main natural enemy, *Anthocoris nemorum*, and whether predation was affected. The herbivore laid more eggs, and the predator survived longer, on female plants in the lab. However, these effects did not translate into differences in predation rates in laboratory experiments or differential insect abundances on plants of either sex or plantation sex composition in the field. Plant genotype did have a significant effect on insect abundances, but this was due to plant traits other than sex. The results indicate that manipulating plant

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sex will not lead to improved biological control or reduced insect herbivory in *S. viminalis* energy forestry, but suggest that a focus on plant genotypic differences offers promise for improving management practices.

## 1. Introduction

Although there is strong evidence that wild plant traits can influence insect predators and parasitoids directly (Wäckers et al. (2013)) and indirectly (Fortuna et al., 2014), studies on the effects of domesticated crop traits on these agents of biological control are scarce, especially in large-scale field experiments. Consequently, rather than optimizing focal crop traits, current Integrated Pest Management (IPM) practices rely on intercropping with companion plants (Bickerton and Hamilton, 2012), flower strips (Tschumi et al., 2015), or other habitat manipulation approaches (Landis et al., 2000) in order to provide biocontrol agents with plant-based food. However, optimising focal crop traits may provide powerful new tools for efficient IPM (Stenberg et al., 2015). As we learn more about the ways in which domestication affects the rewards that predators derive from crops, it is becoming increasingly clear that predators and parasitoids can be affected by the specific cultivar, or cultivar mix, that is used in a plantation (Chen et al., 2015). It is therefore important to identify appropriate plant genetically based traits that can directly reduce herbivory or improve the biological control of herbivorous pests.

Utilizing genetically based plant traits to control pests requires the selection of traits, such as secondary chemistry, herbivore-induced volatiles, nectar, and structural defences, that lead to the desired effects. Plant sex is often an important trait structuring herbivory (Cornelissen and Stiling, 2005) in wild plants. Predation can also be affected by plant sex (Kabir et al., 2014; Mooney et al., 2012), and studies on its potential utility in IPM programs including dioecious crops are therefore merited. Previous studies have shown that wild male and female plants can differ with respect to both resistance and rewarding traits (Bañuelos et al., 2004; Boecklen et al., 1990; Bullock, 1984; Pollard and Briggs, 1984). In concert with other types of trait variation, sex ratios various types of trait variation of crops could therefore be manipulated in domesticated plants to improve crop protection. Intersexual differences in resistance and rewarding traits can affect insect herbivores and can be expected to affect omnivorous natural enemies of the herbivores. One proposed mechanism for male-biased herbivory is that higher reproductive costs for female plants have led to females evolving better defences, because females can derive greater fitness gains from investing resources in defences than through investment in growth (Lloyd and Webb, 1977). However, exceptions to male-biased herbivory in dioecious plants are common (reviewed by Cornelissen & Stiling 2005) and for dioecious species little work has been done on the natural enemies of herbivores that can play an important role in IPM, suggesting that research is needed and sex differences cannot be assumed. Plant nutritional quality may affect herbivores and their omnivorous predators differently, as herbivores are generally limited by nitrogen sources such as amino acids (Mattson, 1980), and because they may feed on different plant tissues (e.g. nectar or leaf tissue). In addition, quality of herbivores as prey may vary with host plant quality (Chen et al., 2015). Pollen and nectar, important food resources for both herbivores and their natural enemies, including both predators and parasitoids, often vary in nutritional value among plants and could therefore influence biological control as well as herbivory, offering opportunities for crop breeding (Stenberg et al., 2015).

Domesticated plant species from the Salicaceae family are commonly used in short rotation forestry as bioenergy crops, but the fact that most species in this family are dioecious (Renner 2014: S1) is largely ignored by breeders and farmers. However, sex-biased (most commonly male-biased) herbivory has been commonly observed, likely due to females being better-defended (Cornelissen and Stiling, 2005), indicating the need for further research on its significance in domesticated crops. In this study we investigate whether using plants of one sex or the other can reduce herbivory (directly, or indirectly via natural enemies) in Short Rotation Coppice of common osier (*Salix viminalis* L.). Short Rotation Coppice is a practice in which an energy forest is repeatedly grown for 3–5 years and harvested, over a period of approximately 20 years. Blue willow beetle (*Phratora vulgatissima* L., Chrysomelidae), the most serious insect pest of willow (*Salix* spp.) and poplar (*Populus* spp.) in Short Rotation Coppice (Peacock et al., 2001), prefers female plants of the wild crop relative *Salix cinerea* in natural populations (Kabir et al., 2014). Notably, it does so even though predation rates on female plants from the common flowerbug (*Anthocoris nemorum* L.), an anthocorid that is an omnivorous predator which feeds on *P. vulgatissima* eggs and *Salix* spp. nectar, are higher (Kabir et al., 2014). Such effects on insect preference and performance indicate that manipulating plant sex may be a powerful component of the IPM toolbox for *Salix*. For example, if higher predation rates reduce pest abundances on plants of either sex, biological control may be improved by using cultivars of that sex only. Another possibility is that mixing male and female plants in the same field (as a means of intra-specific intercropping to increase biodiversity) would promote biocontrol and reduce herbivory. However, an observational study surveying monoculture *S. viminalis* plantations for the effects of plant sex on a number of insect herbivores, albeit excluding the most important pest, *P. vulgatissima*, found no clear effects (Åhman, 1997). It has therefore been unclear whether, and how, *S. viminalis* sex affects herbivory by *P. vulgatissima* and biological control of *P. vulgatissima* by its predator *A. nemorum*.

We investigated whether manipulating plant sex in *S. viminalis* plantations can reduce herbivore damage by *P. vulgatissima* and improve the biological control exerted by *A. nemorum*. To assess herbivory and biological control we planted a large-scale field experiment comparing monosexual male, monosexual female and mixed sex plots, and recorded the abundances of the two insects over three seasons. In addition, we performed a series of greenhouse and laboratory experiments in which we estimated plant sex effects on insect preference, development, survival, consumption, oviposition and predation. Based on previous findings with *P. vulgatissima* and *A. nemorum* (Kabir et al., 2014), we hypothesized that (i) abundances of the herbivore and omnivore should be higher on female *S. viminalis*, and that (ii) monosexual female fields should host greater abundances of both insects than male fields, with mixed-sex fields having intermediate abundances. We further hypothesized that (iii) insect performances would be better on female plants. Consequently, we expected that the herbivore *P. vulgatissima* and predator *A. nemorum* and would (iv) choose female plants in an arena setting, and prefer to (v) feed and (vi) oviposit on female plants.

## 2. Materials and methods

### 2.1. Study species and experimental material

*Salix viminalis* is a woody Salicaceae species used as a bioenergy crop and grown in Short Rotation Coppice in agricultural fields. For economic and environmental reasons, pesticides are not applied to Short Rotation Coppice fields (Stenberg et al., 2010), although biomass reductions of up to 39% due to herbivory have been recorded (Björkman et al., 2000), and experimental mechanical defoliation can reduce shoot diameter at a height of 1 m by as much as 72% (Bell et al., 2006). Both male and female *S. viminalis* produce floral nectar that is consumed by pollinators and omnivorous predators. It is not known