



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



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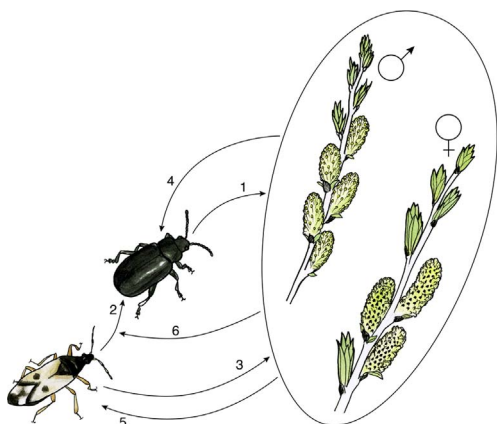
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GRAPHICAL ABSTRACT

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* 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

whether nectar quality of *S. viminalis* to the predator *A. nemorum*, or other consumers, differs between plant sexes. In Sweden, *S. viminalis* is naturalized (Hylander, 1971). *Phratora vulgatissima*, a leaf-chewing chrysomelid beetle, is the most serious pest of *S. viminalis* in energy forestry (Peacock et al., 2001), and finding effective integrated pest management strategies is crucial in reducing losses due to herbivory. The predator, *A. nemorum*, is one of the most commonly occurring enemies of the pest *P. vulgatissima*. *A. nemorum* is an omnivorous true bug (Hemiptera) that emerges in adult form before *P. vulgatissima* adults in spring. It can feed both on *Salix* spp. nectar and on *P. vulgatissima* eggs. *Anthocoris nemorum* has negligible effects as a herbivore on *S. viminalis* (Laustein, 1979), but its survival and egg consumption are affected by host plant genotype (Stenberg et al., 2011).

We used male and female *S. viminalis* clones originally collected from Swedish naturalized populations in our experiments. Cuttings were collected in February 2013 and 2014 from experimental plantations outside Uppsala, Sweden, and kept in -5°C dark rooms prior to planting. All soil used for plants in the greenhouse was sterilized in a microwave oven at 900 W. Pots used in the greenhouse measured 11×11 cm and were 12 cm tall. The greenhouse was kept at 20°C with 18 h of supplemental light and plants were supplied with nitrogen fertilizer approximately once per week. Greenhouse experiments were started six to ten weeks after planting and no more than one experiment was performed in a given greenhouse at any one time. Plant placement in the greenhouse was always randomized.

2.2. Field experiment

To test for the effects of *S. viminalis* sex on the abundances of the herbivore *P. vulgatissima* and the predator *A. nemorum*, we performed a field experiment in which plant sex was used as an individual plant treatment and plant sex composition was used as a plot treatment. In May 2013, we planted two cuttings of each of 20 clones (40 plants) per plot in 30 plots outside Uppsala, Sweden (Latitude: $58^{\circ}83'$, Longitude: $17^{\circ}78'$, Fig. 1.). Plots were randomly assigned to one of three plot treatments (male only, female only or mixed) in a block design with 10 blocks consisting of 3 plots each (Fig. 1). Plots within blocks were spatially adjacent to each other, with an exception for plot 9, which was separated from plot 7 and 8 because of spatial limitations. The plots comprised 20 male clones, 20 females or a balanced mix of 10 randomly selected clones per sex. Because we wanted to minimize insect movement among plots after colonization, we planted with a minimum distance of 180 m between the closest plots. Cuttings were planted in grids at distances of 70 cm, as is common practice in commercial plantations (Moritz et al., 2016), and plots measured 10×10 m. The spatial arrangement of all clones within plots was random. Because all experimental plants experienced roe deer herbivory during the first growing season, we cut the shoots down to the planted cuttings, fenced each plot and let new shoots grow during the growing seasons of 2014 and 2015. To count the abundances of *P. vulgatissima* and *A. nemorum*, we visually scored one randomly selected shoot per plant once in 2014, on three occasions in 2015, and once in 2016.

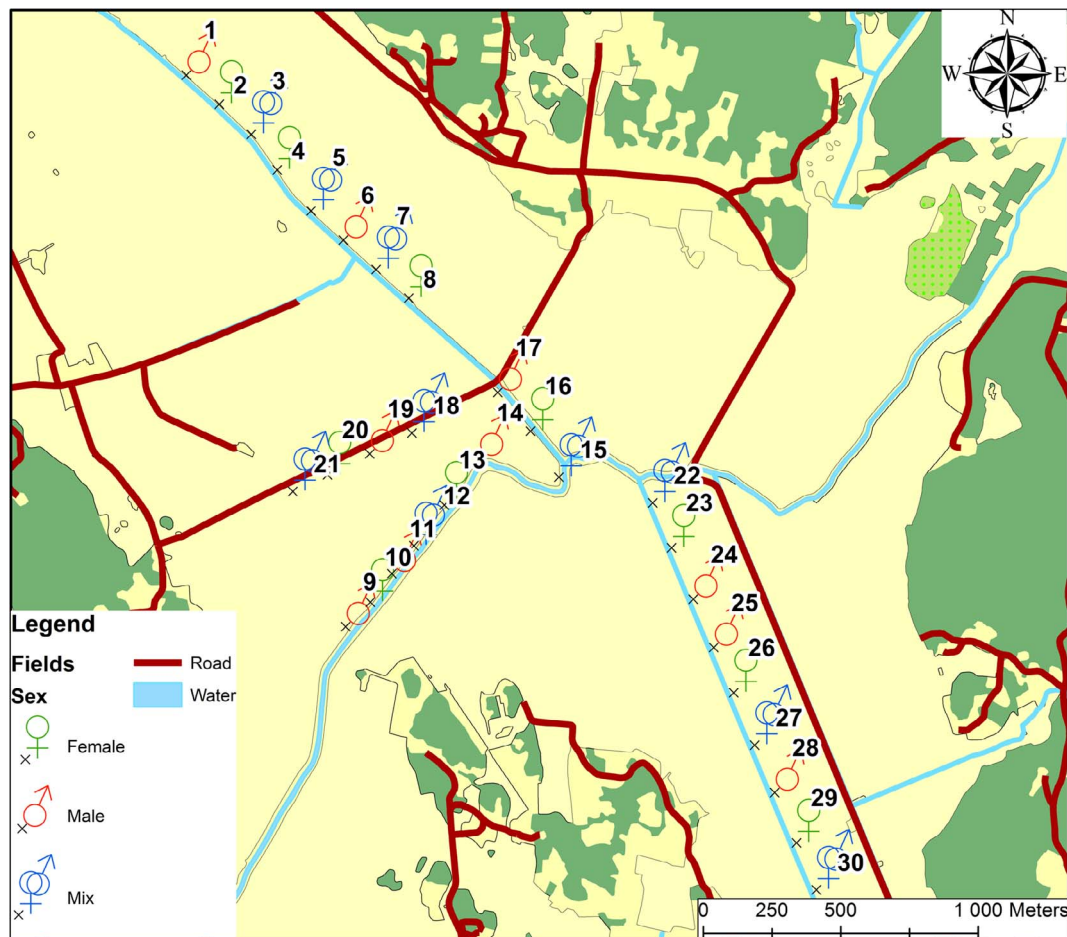


Fig. 1. Map over field experiment, with male and female monosexual plots, and plots comprised of even mixes of male and female genotypes. Two clones of 20 genotypes per plot were used. The map was produced using ArcGis Desktop 10 (ESRI).

2.3. Herbivore choice experiments

To assess which plant sex is more likely to be visited by nearby adult *P. vugatissima* herbivores at first encounter when both plant sexes are present, we performed a choice experiment using a lab population originally collected outside Uppsala, Sweden. In a net cage (base 40 × 40 cm, height 78 cm) we placed one male and one female plant with a Styrofoam “bridge” between them. We released one beetle on the middle of the bridge and recorded whether the beetle was present on the male or female plant 30 min after release. In total 20 male and 20 female plant genotypes were used. Each genotype was used twice but in unique male-female pairs for each replication. We performed 40 replications with unique beetles and male-female pairs in each replication. The bridge was swabbed with ethanol between replicates to remove any traces of volatile compounds left by the beetles.

2.4. Herbivore larval feeding experiment

To test for leaf consumption by leaf beetle larvae not presented with any choice, we placed three larvae hatched during the previous 24 h on a male or female *S. viminalis* leaf in 2014. We used 19 plant genotypes per plant sex, one plant individual per genotype constituting an experimental replicate, collected in 2014 and grown in the same conditions as the plants used in the choice experiments. Each leaf, with three newly hatched *P. vugatissima* larva, was enclosed in a cylindrical (height 4.2 cm, top ø6.9 cm, bottom ø5.6 cm) plastic cup with narrow holes permitting airflow but preventing escape of the larvae. After 48 h the experiment was terminated and each leaf was photographed alongside 0.5 cm² graph paper and the survival, mass and length were recorded for each larva. We analysed the leaf areas consumed using the software package ImageJ by standardizing measurements against the known scale in one photograph per replicate.

2.5. Herbivore oviposition experiment

To assess oviposition preferences, we randomly placed 19 male and 19 female *S. viminalis*, grown as in the choice experiment for six weeks, in a cage (base 60–160 cm, height 108 cm) with mesh net walls, and released 38 leaf beetles (19 of each sex), which were evenly distributed across the corners of the cage. Distances were approximately 3 cm between plant pots and neighbouring plants were in contact with each other. After ten days, we recorded the number of beetles that were present on each plant, counted the number of *P. vugatissima* eggs and photographed each leaf that had been attacked. We quantified herbivory by image analysis as in the feeding experiment.

2.6. Herbivore and omnivore performance experiments

To assess whether the herbivore *P. vugatissima* grew larger and/or faster (i.e. antibiosis) when feeding on *S. viminalis* of either sex, we placed individual larvae on leaves from 20 male and 20 female genotypes and enclosed each in a plastic vial (ø3 cm, 7 cm height) with a perforated lid. Each clone was used for one replicate only. A moist foam base kept humidity at a level where the leaves stayed fresh for at least two days and the larvae did not drown. The vials were randomly placed in growth chambers at 20 °C with 18 h of light per day. The leaf of each vial was replaced every second day and the dates of pupation and reaching the imago stage were recorded. We weighed each larva when it pupated and when it emerged as an adult using a Mettler Toledo MX5 microbalance (0–5.1 g ± 1 µg). We replaced larvae that died before pupation and restarted the experiments for the corresponding plant genotypes.

To assess whether the omnivorous predator *A. nemorum* developed faster and/or survived longer when feeding on *S. viminalis* plants of one sex or the other, we collected branches from 25 female and 21 male genotypes from the clone archives used in other experiments described

in the present study. Twenty-eight of the genotypes were not the same as those used in the field experiment, because other genotypes were more easily collected from nearby clone archives at that time. Because the predator *A. nemorum* primarily forages on *Salix* spp. in spring and early summer, we performed the experiment during the *S. viminalis* flowering season and included only twigs with nectar-producing catkins. We placed the green parts of the twigs in dry plastic mugs (ø9 cm, height 20 cm) with the basal part of each twig exiting through the bottom and entering a similar mug filled with water, through a hole between the mugs, to provide water to the plant while minimizing the risk of the insects drowning (Kabir et al., 2014). The lid of each dry mug was perforated. The replicates were randomly placed in climate chambers as described for the performance experiments on *P. vugatissima*. We released newly hatched *A. nemorum* originating from a population outside of Uppsala, Sweden, and recorded survival after 15, 19 and 26 days.

2.7. Omnivore egg predation experiment

To experimentally test whether plant sex has any effects on predation efficiency (number of eggs consumed over a specified time) by *A. nemorum* on the herbivore *P. vugatissima*, we grew one plant of each of the 19 male and 20 female experimental plant genotypes in greenhouses as described above. Plants were covered with perforated plastic bags so that insects would not escape the experimental setting. We released one mated *P. vugatissima* female per plant and recorded the first day of oviposition, after which we released one adult *A. nemorum* predator on each plant. Number of eggs, and clutches laid per day by the herbivore varied among replicates but there was never a complete egg predation. Consumed and intact eggs are easily distinguished based on appearance (Stenberg et al., 2011), and we counted numbers of intact and consumed eggs one week after introduction of *A. nemorum*. We terminated the experiment when the *A. nemorum* individuals died, and counted the number of days that each *A. nemorum* survived.

2.8. Statistical testing

All statistical testing was performed using the statistical software package R (R Development Core Team, 2015). To test for the effects of plant sex and plot sex treatment on the abundances of adult *P. vugatissima* herbivores and *A. nemorum* predators, we used the *glmer* function in the lme4 package (Bates et al., 2014) to construct generalized linear models with a Poisson family and a log link. Insect abundances, in 2014 and 2015 for *P. vugatissima* and in 2014 for *A. nemorum*, were used as response variable, and plant sex and plot sex treatment were used as fixed factors to capture effects of individual and neighbourhood sex effects. Date, block, plot nested within block and position within plot were used as random factors. For the *P. vugatissima* abundances, a repeated measures design was used, where *S. viminalis* individual was used as a random factor. The same model was used to test for effects on *P. vugatissima* larvae. Because *A. nemorum* was only present at one occasion, no repeated measurement term was necessary for modelling its abundance.

We tested for *P. vugatissima* plant sex choice with a χ^2 -test. In two cases, no plant was chosen, and we excluded these replicates from the analysis.

To assess the effects of plant sex on *P. vugatissima* larval feeding area (mm²), we used a linear model with the area consumed as a response variable, and plant sex and number of surviving larvae as fixed factors. To test for effects of plant sex on *P. vugatissima* adult feeding area (mm²), we used a *t*-test.

We used a Generalized Linear Model with a Poisson error family and log link to test for plant sex effects on *P. vugatissima* oviposition, both in the herbivore oviposition experiment (2.5) and the omnivore egg predation experiment (2.7). Egg number was used as response variable and plant sex as factor.

To test for the effects of plant sex on performance, we also used *t*-tests to compare development time (days) and weight (mg) at the pupal and adult stages for *P. vulgatissima* that had been fed male and female leaves. In order to test for the effects on *A. nemorum* survival over time, as a measure of performance, we used a generalized linear mixed model with a binomial error family and a log link, in which survival was used as a response variable, plant sex as a fixed factor and time as a random variable.

Differences in *A. nemorum* egg predation rate between male and female plants were tested in a generalized linear model with a Poisson distribution and a log link, with eggs consumed being used as a response variable, plant sex as a fixed factor and total number of eggs as a random variable. We used a *t*-test to test for differences in *A. nemorum* survival when bagged with *P. vulgatissima* eggs on male and female plants.

3. Results

3.1. Field abundances

In the field we did not observe any differences in insect abundances between plants of different sexes or among the sex treatments (male, mix or female) of plots. The abundance of adult *P. vulgatissima* herbivores did not differ between plants of different sexes ($\chi_2 = 0.29$, $P = 0.59$, d.f. = 1), or among plot sex treatments ($\chi_2 = 0.30$, $P = 0.86$, d.f. = 2), over the 2014, 2015 and 2016 field seasons (Fig. 2). There were also no differences in abundance of *P. vulgatissima* larvae between sexes ($\chi_2 = 0.17$, $P = 0.68$, d.f. = 1) or among plot sex treatments ($\chi_2 = 0.12$, $P = 0.94$, d.f. = 2). In total, we found 41 *P. vulgatissima* herbivores in July 2014, 42, 17 and 5 individuals in June, July and August 2015 respectively, and 74 in June 2016. The abundances of *Anthocoris nemorum* predators were also unaffected by plant sex ($\chi_2 = 0.60$, $P = 0.44$, d.f. = 1) or plot sex treatments ($\chi_2 = 2.11$, $P = 0.35$, d.f. = 1) in 2014, when, in total, 23 individuals were found. In June, July and August 2015, and in June in 2016, no *A. nemorum* were found in field plots.

3.2. Herbivore choice experiments

Phratora vulgatissima did not show preferences for either sex in our cafeteria experiment ($\chi_2 = 1.6$, $P = 0.21$, d.f. = 1). Twenty-three beetles were recorded on female plants and 17 on male plants.

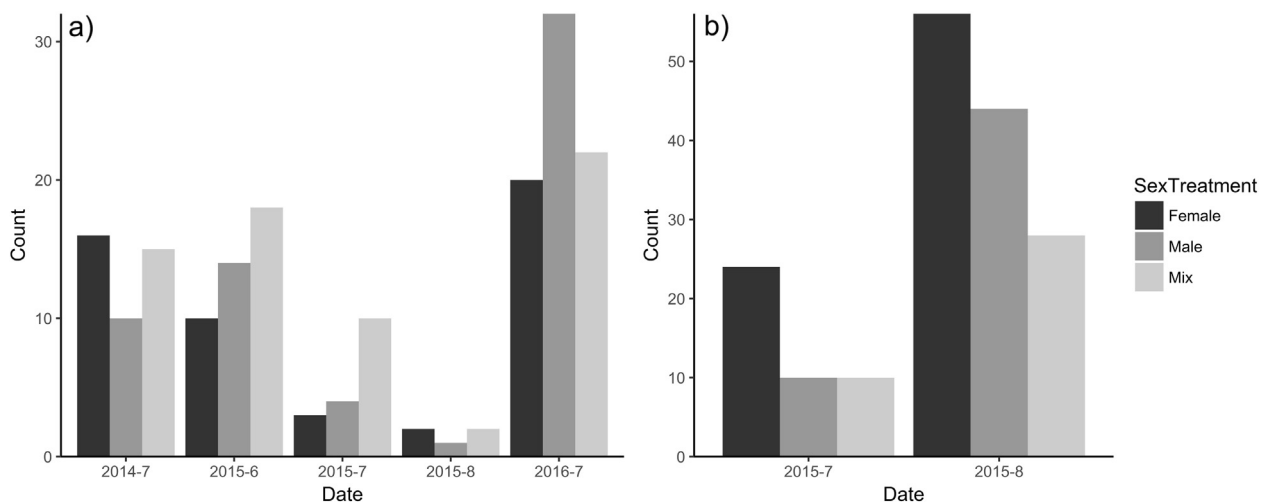


Fig. 2. Total abundances did not differ among female, male and mixed sex plots of *Phratora vulgatissima* a) adults and b) larvae at sampled occasions.

3.3. Larval and adult herbivore (choice and non-choice) feeding experiments

We found no effects of plant sex on the extent to which *P. vulgatissima* herbivores fed on *S. viminalis*. *Phratora vulgatissima* larvae that were raised on *S. viminalis* leaves did not consume different areas of leaves on the two plant sexes ($t = 0.89$, $P = 0.38$, d.f. = 1). Adult beetles fed with designated plant genotypes did not consume different leaf areas on male and female plants ($t = -1.18$, $P = 0.24$, d.f. = 1) when they were fed only one genotype. In addition, *P. vulgatissima* did not feed more on plants of one sex or the other when presented with a choice ($t = -0.93$, $P = 0.36$).

3.4. Herbivore oviposition experiment

Mated female *P. vulgatissima* herbivores preferred to oviposit on female *S. viminalis* when presented with a choice ($z = -2.05$, $P = 0.04$, d.f. = 1), but they did not feed more ($t = 0.93$, $P = 0.36$, d.f. = 1), and were not more frequently present, on plants of one sex or the other ($z = -0.24$, $P = 0.82$, d.f. = 1). In total, 285 eggs were found on female plants and 238 on male plants.

3.5. Herbivore and omnivore performance experiments

Effects of plant sex on performance differed between the two insect species. *Anthocoris nemorum* predators showed higher survival over time when feeding on flowering *Salix viminalis* female twigs than on flowering male twigs ($\chi_2 = 3.88$, $P = 0.05$, d.f. = 1, Fig. 3). However, plant sex had no effect either on duration of development until pupation ($F = 0.22$, $P = 0.64$, d.f. = 1) or the imago stage ($F = 0.54$, $P = 0.47$, d.f. = 1), or on weight of the pupal ($F = 3.26$, $P = 0.08$, d.f. = 1) or adult ($F = 0.009$, $P = 0.93$, d.f. = 1) form of *P. vulgatissima* herbivores. Furthermore, survival up to the imago stage for *P. vulgatissima* was unaffected by plant sex.

3.6. Omnivore egg predation experiment

We found no effects of plant sex on adult *A. nemorum* in the predation experiment with three trophic levels. The risk of a *P. vulgatissima* egg being eaten by *A. nemorum* in the predation experiment was not affected by plant sex ($t = 0.31$, $P = 0.76$, d.f. = 1). Furthermore, *P. vulgatissima* egg laying ($F = 0.37$, $P = 0.95$, d.f. = 1) and clutch size ($F = 0.00$, $P = 0.97$, d.f. = 1) in the presence of *A. nemorum* did not differ between the plant sexes. The longevity of *A. nemorum* in this experiment did not differ between plants of different sexes ($t = 0.48$,

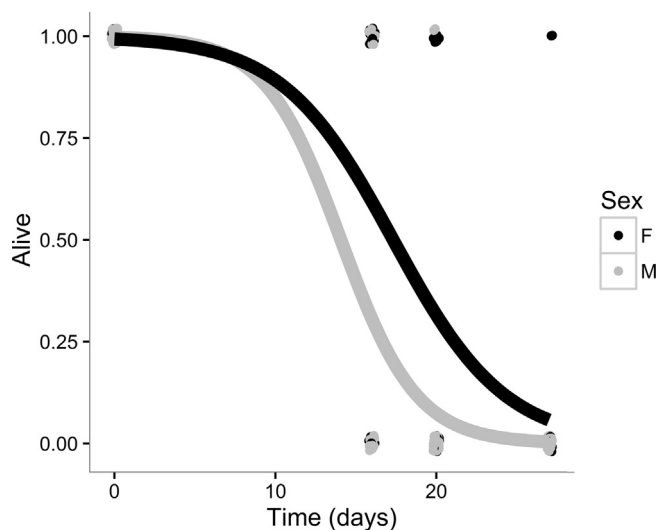


Fig. 3. Proportion of surviving predators (*Anthocoris nemorum*) over time in the performance experiment where insects were confined to one genotype, was higher in replicates with female host plants.

$P = 0.63$, d.f. = 1).

4. Discussion

Salix viminalis is a bioenergy crop for which breeding has begun only in the last few decades. Being early in the breeding process, the potential for including traits other than those directly related to growth is high. Knowing that this bioenergy crop suffers substantially from heavy insect damage and insecticide treatment is no option it is important to identify plant genetic material that influences interactions with herbivores and natural enemies in desirable ways. In this study, we investigated how traits of commercial *S. viminalis* may influence natural herbivore resistance and biological control by evaluating the effects of plant sex on the field abundances and abundances, behavior, performance, and fitness of an insect pest species and an omnivorous insect predator. We found that female *S. viminalis* plants were preferred over males for oviposition by the main insect pest, *P. vulgatissima*. This contrasts with previous results for wild *S. cinerea* (Kabir et al., 2014), where neither plant sex was preferred for oviposition, and indicates that sex-biases are likely plant species-specific. For example, differences between these two *Salix* species with respect to leaf morphology and/or defences may have caused plant species-dependent patterns in plant sex-biased insect preferences.

Although *S. viminalis* females were preferred for herbivore oviposition in one of the experiments in the present study, we found that insect field abundances and performance-related behavior and traits were unaffected by plant sex. While our results suggest that female *S. viminalis* plants may be preferred as hosts, there was no plant sex difference in potential number of eggs per plant and beetle. It is possible, however, that undetected oviposition-related effects of sex were present in our experiments. Because herbivore eggs are distributed over clutches that often represent oviposition events, comparing also the numbers of egg clutches between treatments may provide additional information (Stephan et al., 2015) of plant sex preferences. We did not record number of clutches and thus could not analyse effects on number of clutches.

Phratora vulgatissima adults may disperse among plants, but their larvae typically remain on their natal host plant. Therefore initial choice of the first adult generation of a year, and oviposition preferences, may be crucial with respect to the subsequent leaf damage caused by larvae. However, abundances were nevertheless similar between field treatments, suggesting that there are no strong effects of plant sex over larger spatial scales. The abundances and predation rates

of the predator (*A. nemorum*) were similar between plants of different sexes, and between fields with different sex ratios. This result indicates that predation rate has not led to *P. vulgatissima* abundances being balanced between plant sexes or fields with different plant sex ratios. We did not measure the abundances of other potential predators, but we only rarely observed *Orthotylus marginalis* (KKM, personal observation), another major natural enemy of *P. vulgatissima*. Given the scale of our field experiment, it is unlikely that any major effects of plant sex on insect abundances would have been missed. However, it is possible that there may have been undetected differences during times where we did not record abundances and higher overall abundances likely would have led to higher detectability. Overall abundances being rather low may also have affected detectability of sex-biases, because *P. vulgatissima* is an aggregating species. In addition, plant ages differed among our experiments, where field plants were up to four years old and laboratory plants were as young as six weeks at the time of exposure to insects. Herbivory may vary with age or life stage in plants (Avila-Sakar and Romanow, 2012), and it is possible that this introduced variation to our experiments. However, plants with ages spanning across the ages of plants used in our experiments have previously successfully been used to demonstrate genotypic effects on life history traits of *P. vulgatissima* (Torp et al., 2013) and *A. nemorum* (Stenberg et al., 2010), suggesting that we captured plant ages that can affect these insects.

Our laboratory and field experiments provided mixed evidence for plant sex effects on *A. nemorum* predators. While survival of *A. nemorum* was higher on flowering *S. viminalis* female clones than on males, there was no difference in survival time on bagged plants where *P. vulgatissima* eggs were available. However the insects in the latter experiment were not all of the same age at its start, which may have introduced variation in survival rate. Our results contrast with the shorter development time found on vegetative *S. cinerea* male plants compared to female plants (Kabir et al., 2014). Effects of willow sex on *A. nemorum* could depend on plant species, seasonality plant age and/or host prey. Host prey availability as a modulator of host plant quality is supported by the finding that *A. nemorum* prefers female plants, despite performing better on vegetative male plants, of *S. cinerea* (Kabir et al., 2014). Potential differences between *S. viminalis* and *S. cinerea* leading to different effects on *A. nemorum* and *P. vulgatissima* may include secondary metabolite content, structural defences and evolutionary history.

Species-specificity and variation in plant sex effects on herbivory and predation, as discussed above, may be important when considering plant sex effects in other crops, or wild plants. If common denominators for different types of plant sex effects can be identified however, plant sex effects may be better understood and more promising as enhancers of plant protection in dioecious crops. Causal traits in plant sex-biased interactions may include plant sexual dimorphism in chemical or structural defences (Ågren et al., 1999), or nutritional values in plant tissues consumed by the herbivore or potential biological control agents.

Our laboratory results should be interpreted with caution, especially in the light of little evidence for differences. The statistical power our laboratory experiments was to some extent sacrificed for the benefit of the broad variety of approaches that we used (Supplementary material 1). Our rationale was to prioritize the number of investigated mechanisms for differences in herbivore and predator performance between *S. viminalis* sexes over high power for detecting weak effects between sexes. Because our main goal of this study was to detect effects on a broad set of traits with potential relevance to plant breeding, we sought differences with large effect sizes as these have higher practical significance (Kotrlík and Atherton, 2011). Thus, we do not suggest that there is conclusively no difference in herbivory or predation between the sexes of *S. viminalis* and more extensive studies may have found weak effects that our study missed. However, these weak effects would be unlikely to be useful in an IPM context.

Our results suggest that, for the pest and its main natural enemy,

plant sex is not a major element in the community genetics of *S. viminalis*. This contrasts with the findings of our previous studies showing that plant sex is important for a major rust pathogen that attacks *Salix* Short Rotation Coppices (Moritz et al., 2016). The rate of infection by the rust was significantly higher on female plants, suggesting that farmers should select male cultivars in order to reduce disease incidence. Similarly, we have also shown that a mix of plant sexes increases herbivory by roe deer (Moritz, 2017), implying that plant sex may still be an important component to take into consideration in crop production and management. In addition, reports of sex-biased herbivory in other managed dioecious plants (e.g. *Acer negundo*, Jing and Coley, 1990) underline the importance of plant sex for trophic interactions and strengthening the case for potential utilization of plant sex in breeding programmes. However, there are also other examples of dioecious plants not experiencing plant sex-biased herbivory, such as *Pistacia lentiscus* (Hjältén et al., 1993). Other potential candidates for sex biased effects in cultivated plants may for example include poplars (*Populus* spp.), spinach (*Spinacia oleracea*), and papaya (*Carica papaya*). Because the present study shows that plant sex has no important effects on the biocontrol of pest insects in *S. viminalis* it, however, suggests that female cultivars and plant sex mixes can safely be avoided in order to reduce pathogen attack and roe deer problems, without compromising insect pest management. Rather, our results suggest that a focus on developing plant traits that confer resistance to the herbivore *P. vulgarissima* and, preferably simultaneously, promote omnivorous predators will be the best IPM strategy. Our finding that field abundances were dependent on plant genotype increases the likelihood of success by providing opportunities to identify particularly suitable plant traits or genotypes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocontrol.2017.09.006>.

References

- Ågren, J., Danell, K., Elmqvist, T., Ericson, L., Hjältén, J., 1999. Sexual dimorphism and biotic interactions. Springer-Verlag, Berlin Heidelberg Germany, pp. 217–246.
- Åhman, I., 1997. Growth, herbivory and disease in relation to gender in *Salix viminalis* L. *Oecologia* 111, 61–68.
- Avila-Sakar, G., Romanow, C.A., 2012. Divergence in defence against herbivores between males and females of dioecious plant species. *Int. J. Evol. Biol.* 2012 897157–897157.
- Bañuelos, M.-J., Sierra, M., Obeso, J.-R., 2004. Sex, secondary compounds and asymmetry. Effects on plant–herbivore interaction in a dioecious shrub. *Acta Oecologica* 25, 151–157.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48.
- Bell, A.C., Clawson, S., Watson, S., 2006. The long-term effect of partial defoliation on the yield of short-rotation coppice willow. *Ann. Appl. Biol.* 148, 97–103.
- Bickerton, M.W., Hamilton, G.C., 2012. Effects of intercropping with flowering plants on predation of *Ostrinia nubilalis* (Lepidoptera: Crambidae) eggs by generalist predators in bell peppers. *Environ. Entomol.* 41, 612–620.
- Björkman, C., Höglund, S., Eklund, K., Larsson, S., 2000. Effects of leaf beetle damage on stem wood production in coppicing willow. *Agric. For. Entomol.* 2, 131–139.
- Boecklen, W.J., Price, P.W., Mopper, S., 1990. Sex and drugs and herbivores: sex-biased herbivory in arroyo willow (*Salix lasiolepis*). *Ecology* 71, 581–588.
- Bullock, S.H., 1984. Biomass and nutrient allocation in a neotropical dioecious palm. *Oecologia* 63, 426–428.
- Chen, Y.H., Gols, R., Benrey, B., 2015. Crop domestication and its impact on naturally selected trophic interactions. *Annu. Rev. Entomol.* 60, 35–58.
- Cornelissen, T., Stiling, P., 2005. Sex-biased herbivory: a meta-analysis of the effects of gender on plant-herbivore interactions. *Oikos* 111, 488–500.
- Fortuna, T.M., Eckert, S., Harvey, J.A., Vet, L.E.M., Müller, C., Gols, R., 2014. Variation in plant defences among populations of a range-expanding plant: consequences for trophic interactions. *New Phytologist* 204, 989–999.
- Hjältén, J., Åström, M., Åberg, E., Danell, K., 1993. Biased sex ratios in Spanish populations of *Pistacia lentiscus* (Anacardiaceae): the possible role of herbivory. *Anales del Jardín Botánico de Madrid* 51, 49–53.
- Hylander, N., 1971. *Prima loca plantarum vascularium Sueciae: Plantae spontaneae vel in tempore recentiore adventitiae*. Första litteraturuppgift för Sveriges vildväxande kärlväxter jämte uppgifter om första svenska fynd. *Svensk Botanisk Tidskrift* 64, 1–322.
- Jing, S.W., Coley, P.D., 1990. Dioecy and herbivory: the effect of growth rate on plant defense in *Acer negundo*. *Oikos* 58, 369–377.
- Kabir, M.F., Mortz, K.K., Stenberg, J.A., 2014. Plant-sex-biased tritrophic interactions on dioecious willow. *Ecosphere* 5, 1–9.
- Kotrlík, J.W., Atherton, J.C., 2011. Reporting and interpreting effect size in quantitative agricultural education research. *J. Agric. Educ.* 52, 132–142.
- Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu. Rev. Entomol.* 45, 175–201.
- Lauestein, G., 1979. Zur Aufnahme von Pflanzensubstanz durch die rauerberische Blumenwanze *Anthocoris nemorum* (Hem.: Heteroptera). *Entomophaga* 24, 431–441.
- Lloyd, D.G., Webb, C.J., 1977. Secondary sex characters in plants. *Bot. Rev.* 43, 177–216.
- Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. *Annu. Rev. Ecol. Syst.* 11, 119–161.
- Mooney, K.A., Fremgen, A., Petry, W., 2012. Plant sex and induced responses independently influence herbivore performance, natural enemies and aphid-tending ants. *Arthropod-Plant Interact.* 6, 553–560.
- Moritz, K.K., 2017. 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. Department of Ecology. Swedish University of Agricultural Sciences, Uppsala.
- Moritz, K.K., Björkman, C., Parachnowitsch, A.L., Stenberg, J.A., 2016. Female *Salix viminalis* are more severely infected by *Melampsora* spp. but neither sex experiences associational effects. *Ecol. Evol.* 6, 1154–1162.
- Peacock, L., Lewis, M., Herrick, S., 2001. Factors influencing the aggregative response of the blue willow beetle, *Phratora vulgarissima*. *Entomologia Experimentalis Et Applicata* 98, 195–201.
- Pollard, A.J., Briggs, D., 1984. Genecological studies of *Urtica dioica* L. III. Stinging hairs and plant-herbivore interactions. *New Phytologist* 97, 507–522.
- R Development Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Stenberg, J.A., Heil, M., Åhman, I., Björkman, C., 2015. Optimizing crops for biocontrol of pests and disease. *Trends Plant Sci.* 20, 698–712.
- Stenberg, J.A., Lehrman, A., Björkman, C., 2010. Uncoupling direct and indirect plant defences: novel opportunities for improving crop security in willow plantations. *Agric. Ecosyst. Environ.* 139, 528–533.
- Stenberg, J.A., Lehrman, A., Björkman, C., 2011. Plant defence: feeding your bodyguards can be counter-productive. *Basic Appl. Ecol.* 12, 629–633.
- Stephan, J.G., Stenberg, J.A., Björkman, C., 2015. How far away is the next basket of eggs? Spatial memory and perceived cues shape aggregation patterns in a leaf beetle. *Ecology* 96, 908–914.
- Torp, M., Lehrman, A., Stenberg, J.A., Julkunen-Tiitto, R., Björkman, C., 2013. Performance of an herbivorous leaf beetle (*Phratora vulgarissima*) on *Salix* F2 hybrids: the importance of phenolics. *J. Chem. Ecol.* 39, 516–524.
- Tschumi, M., Albrecht, M., Entling, M.H., Jacot, K., 2015. High effectiveness of tailored flower strips in reducing pests and crop plant damage. *Proc. R. Soc. B: Biol. Sci.* 282, 20151369.
- Wäckers, F.L., van Rijn, Paul Cornelis Jacobus, Bruin, Jan, 2013. *Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and its Applications*. Cambridge University Press.