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Uncoupling direct and indirect plant defences: Novel opportunities for improving crop security in willow plantations

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ABSTRACT

Increased cropping security, i.e. minimized risk for detrimental stress events and hence improved yield stability, may be achieved by selecting resistant plant genotypes. However, strong direct defences against herbivores have been associated with negative effects on the natural enemies, resulting in weak indirect defences through these plant "bodyguards". We compared the preference and performance of the most detrimental herbivore, the leaf beetle *Phratora vulgatissima*, and a biocontrol agent, the predatory bug *Anthocoris nemorum*, on four willow (*Salix*) genotypes used in short rotation coppicing. The biocontrol agent is omnivorous and survives on the plant in the absence of prey, without causing any apparent damage to the plant. Two of the genotypes intrinsically attracted and supported the bodyguard, and were at the same time partially capable of deterring and resisting the herbivore. In contrast, the third willow clone exhibited very limited direct and indirect resistance, while the fourth clone displayed intermediate levels of both. Thus, direct and indirect defence against herbivores need not be traded off in this system, but may be intertwined, resulting in "super plants" with higher probability of resisting detrimental herbivores, opening up novel possibilities for increased cropping security, without the use of insecticides.

1. Introduction

Herbivores are generally more prone to outbreak in monocultures than in natural systems (Dalin et al., 2009), and may cause severe losses, especially in low-value or organic crops where insecticides are not used. In order to reduce herbivore damage, it is desirable to develop plant genotypes that are intrinsically more resistant to herbivores (direct defence) or that support biological control agents (indirect defence). Intertwining the two defence strategies would probably lead to a more sustainable resistance in the focal plant.

Discouraging enough, ecologists have long presumed that there should be an intrinsic trade-off between direct and indirect defences in plants, since both require resources derived from the plant's metabolic budget (see the *Optimal Defence Hypothesis*, e.g. Price et al., 1980; Ode et al., 2004). If, however, one disregards examples involving herbivore-mediated effects of plant quality on parasitoids, the literature is rather scarce, and evidence for trade-offs between direct and indirect defence is mainly limited to plants that produce extra-floral nectar (e.g. Rehr et al., 1973; Rudgers et al., 2004).

Trade-offs may also arise when plant traits important for attracting and supporting bodyguards also are favourable for herbivores. For example, many bodyguards are omnivorous and receive important nutrients from the same green plant tissue that the herbivores utilize (Coll, 1996; McMurtry and Croft, 1997; Agrawal et al., 1999; Eubanks and Denno, 2000; Coll and Guershon, 2002; Rodriguez-Saona and Thaler, 2005; Groenteman et al., 2006). However, the relation between pest insect vs. bodyguard responses to plant genotype has almost entirely been overlooked. Agrawal et al. (1999) and Eubanks and Denno (2000) simultaneously studied the response of herbivores and predators to their shared host-plant and suggested that bodyguards and pest herbivores should respond similarly to shared plant resources (Agrawal et al., 1999; Eubanks and Denno, 2000). We feel, however, that their suggestion may apply more to their specific systems (i.e. cotton and lima bean) rather than having universal significance. Here, we hypothesize that direct and indirect defences may be entangled in more complicated ways. If, for example, the nutritional requirements of pest herbivores and omnivorous bodyguards are different this may open up a possibility for breeders to disassociate the two defence strategies and optimise both of them independently.

Rather than looking at direct and indirect defence as two contrasting strategies being subject to trade-off between each other we propose that these two defences are two indispensable aspects

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of the system, which together underpin an integrated and balanced defence package. Based on empirical evidence and logical reasoning, we argue that in perennial crops it is wise to balance the investment in direct and indirect defences in such a way that they co-vary.

In this paper we describe a study of the direct and indirect defences of different *Salix* (willow) clones against the blue willow beetle, *Phratora vulgatissima* (Coleoptera: Chrysomelidae), which is a severe pest in European coppicing willow plantations (e.g. Bell et al., 2006). *Phratora* damage may, however, be reduced by predatory omnivorous bugs such as *Anthocoris nemorum* (Heteroptera: Anthocoridae), which is considered to be an important bodyguard both for *Salix* and other plants (e.g. Björkman et al., 2003, 2004). In past breeding programs, the focus has been on direct plant resistance, since existing hypotheses regarding trade-offs have precluded the possibility of simultaneously optimizing direct and indirect defences (Kelly and Curry, 1991; Glynn et al., 2004). Here, we investigated bodyguard attraction to, and fitness on, different clones of *Salix*, in order to obtain reliable estimates of the clones' levels of indirect defence. Simultaneously, herbivore attraction to, and reproduction on, the *Salix* clones were investigated.

2. Materials and methods

2.1. Study species

2.1.1. Plants

Willows are grown in monocultures for biomass production in which the use of pesticides is not defendable neither from environmental, nor economic reasons. Due to the frequent hybridization and polyploidization in the genus *Salix*, variation within and among species may be high and overlapping; this opens up good opportunities to find genotypes with traits suitable for various conditions. It also means that the species concept is problematic – thus clone is often a more relevant identity than species. This taxonomical difficulty is less of a problem in practical bioenergy forestry. Here we chose to work with four clones of *Salix*, two of which are available from Lantmännen Agroenergi AB: Gudrun and Loden (*S. dasyclados*), and two breeding clones: 78-0-183 and 78-0-21 (*S. viminalis*). The specific clones used here were selected as they display phenotypic variation in traits relevant to *Phratora* and, thus, seem to represent "extremes" in a more or less continuous variation. In addition there is ongoing and existing research on these clones in sister projects.

2.1.2. Herbivore

Phratora vulgatissima L. is the most important defoliator of *Salix* in Europe and has a broad distribution across Europe and Asia. It is restricted to *Salix* and both adults and larvae skeletonize the leaves (Peacock and Herrick, 2000; Peacock et al., 2001). Overwintering takes place outside *Salix* plantations in vertical objects that can provide shelter, for example reeds or trees with ageing bark (Björkman and Eklund, 2006). The overwintering adults emerge in April and feed on the young *Salix* leaves for about two weeks before mating and subsequent egg-laying. Larvae feed on the leaves for about 18–30 days, depending on leaf quality and temperature, and then they pupate in the ground. Adults emerge after about two weeks and feed on the leaves before hibernation. Generally, *Phratora* has only one generation per year in Sweden. For this study, we collected *Phratora* individuals from known overwintering sites near Uppsala, Sweden, during late February and kept them in a refrigerator at 5 °C until the beginning of the experiment.

The omnivorous predatory bug *Anthocoris nemorum* L. is widely distributed across Europe and Asia. It can be found in large numbers not only on *Salix*, but also on, among other plants, apple trees and nettles. *Anthocoris* overwinters as an adult and becomes active as soon as the temperature rises in the spring. First instar nymphs have been observed sucking plant fluid from leaves and twig bases, which indicates that vegetable matter may be a very important food source for nymphal survival (personal observation). It is, however, very important to note that although *Anthocoris* may be nourished by *Salix*, and in extreme situations even completes its development without animal food, previous studies have shown that plant-feeding *Anthocoris* do not reduce plant fitness (Lauenstein, 1979). Thus, any direct cost of anthocorid feeding on *Salix* should be incommensurably lower than the cost of *Phratora* grazing, which may reduce *Salix* growth with up to 40% (Björkman et al., 2000a). Older anthocorid individuals are predominantly carnivores. *Anthocoris* is an active and aggressive hunter and has been previously shown to attack more egg batches than other bodyguards in *Salix* stands (Björkman et al., 2003). Apart from eggs, it may also prey upon small larvae, but it does not typically attack last instar *Phratora larvae* or adult beetles. *Anthocoris* eggs are laid inside green plant material such as leaves or stems, including *Salix*. The species may have one or a few overlapping generations per summer. For this study we collected *Anthocoris* adults from stinging nettle (*Urtica dioica*) and first instar nymphs from *Salix cinerea* and kept them on green beans at room temperature until the beginning of the experiment.

It should further be noted that *Phratora* and *Anthocoris* have different modes when they feed on *Salix* leaves. While *Phratora* defoliates the leaves, causing significant visible damage, *Anthocoris* pierces and sucks fluid from the leaves, causing no readily apparent damage. The two species may, therefore, be exposed to fundamentally different chemical and physiological plant characters.

2.2. Herbivore preference

On June 17, sixteen 50 cm tall potted *Salix* plants of each of the two clones Gudrun and 78-0-183 were placed at random in a backyard of the university campus, to allow natural colonization by *Phratora*. The distance between neighboring plants was about 1 m. On August 25, the leaf area that had been consumed by naturally colonizing *Phratora* (adult and larval grazing) was scored visually for the two clones. A one-way ANOVA, with clone as a fixed factor, was performed to analyze the data. The statistical software package R 2.7.2 was used for all tests.

2.3. Herbivore performance: egg production

Pairs (♀♂) of *Phratora* were placed in plastic jars and fed with detached leaves from one of the four *Salix* clones. Eggs were counted and leaves were replaced every second day. Because males may stress the females and negatively affect the oviposition rate, each male was removed as soon as egg-laying commenced. Each female was monitored until she died or until no eggs had been laid during two consecutive scoring events. Plant material for this experiment was planted six weeks before the start of the experiment and used for feeding throughout the experiment. Thus, the beetles experienced continuously ageing plants, but leaves were of the same relative age, i.e. leaves from the top 10 cm and bottom 20 cm were not used. Moreover, each individual plant was only used for two consecutive egg counts. Twenty *Phratora* pairs were used for each clone. An ANOVA with *clone* as the fixed factor and *total egg production* as the dependent variable was used to assess direct resistance of the different clones. Individual females that did not produce any eggs at all were removed from the analysis. In addition, an exact binomial test (Crawley, 2002) was used to examine whether the ratio of females failing to produce any eggs at all differed between clones.