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Relation between leaf rust (*Melampsora epitea*) severity and the specific leaf area in short rotation coppice willows

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Abstract Willow leaf rust (*Melampsora epitea*) is an important leaf pathogen in short rotation coppice plantations. Although ecological factors influencing rust severity are rather well known, to date, the connections between leaf morphological traits and rust infections are poorly studied. The aim of this study was to examine the relation between rust severity and specific leaf area (SLA). Willow leaves of three different clones from waste-water-irrigated and non-irrigated plantation areas were used. The results revealed a significant positive correlation between SLA and rust severity. There were clone specific differences whereas more rust pustules were always accompanied with higher SLA. Waste-water-irrigated plants had higher SLA and always more rust damages. These results suggest that leaves with low SLA are less susceptible to leaf rust in various conditions.

Keywords Rust infection · SLA · *Salix* sp.

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Introduction

The use of biomass from fast-growing tree species in a short rotation coppice (SRC) is one of the options for renewable energy production. In temperate zone, the most suitable trees for SRC are poplars (*Populus* sp.) or willows (*Salix* sp.) since they can both achieve high photosynthesis capacity and fast regeneration after coppicing (e.g. Karp and Shield 2008). These plantations differ in many ways from natural forest stands since typical SRC consists of limited number of genotypes over large areas and the density of plants is high (13, 000–20, 000 plants per ha). These factors influence microclimatic conditions and microbial community in the plantation.

Since the high biomass production is essential in SRC, it is important to determine which factors could diminish the plant growth or change the allocation of biomass into various parts of the plants. In addition to nutrient and water availability, plant pathogens and pests can affect the plant growth. One of the most important diseases of SRC willow is the leaf rust caused by *Melampsora*, which infects both poplar and willow plants (Royle and Hubbes 1992). It has been reported that the rust disease can markedly reduce the willow biomass yield (e.g. Verwijst 1993) and therefore rust resistance is a critical criterion for breeding new varieties for SRC. Better knowledge about ecological and morphological factors that have influence on the severity of this disease enables a focus on planting material with desirable characteristics to reduce disease development in SRC.

To date, some information is available on various ecological factors that could influence the severity of *M. epitea*, the most important species of willow rusts. It has been reported that rust susceptibility is species and clone specific (e.g. Åhman 1998), increases during the vegetation period (e.g. McCracken and Dawson 1992) and depends greatly on weather conditions (Johansson and Alström 2000). It was also demonstrated that rust levels are lower when different willow clones and species are grown in mixtures compared to monocultures (McCracken and Dawson 1998; Begley et al. 2009). Some data show that fertilization and irrigation or their combination could increase the rust susceptibility of willow or poplar plants (Orians and Floyd 1997; Coyle et al. 2006; Toome et al. 2009), whereas other studies have not found clear connections between these characteristics (Heiska et al. 2007; Åhman and Wilson 2008). There is strong evidence that a higher canopy density of SRC is accompanied with more severe rust damages on willow leaves (Toome et al. unpublished). Although ecological factors influencing rust susceptibility are rather well studied, there is only limited knowledge about the effect of plant morphological characteristics on this pathogen. Willow leaves may become more susceptible to leaf rust with leaf aging and the infection always starts from lower leaves (e.g. McCracken and Dawson 1992). A study with poplar leaves also demonstrates that leaves with higher wettability are more infected with leaf rust (Pinon et al. 2006).

A common leaf characteristic that describes leaf morphology is specific leaf area (SLA); the ratio between leaf area and leaf mass. This indicator is strongly related to leaf nitrogen content and thus describes well the photosynthetic ability of the leaf (Al Afas et al. 2005, 2007; Schulze et al. 2005). Therefore, at similar growth conditions, SLA can be used to estimate tree growth (Marron et al. 2007). Specific leaf area varies among plant genotypes and within each genotype it depends on the amount of light reaching the leaf. Leaves on top of the canopy, receiving more light, have lower SLA whereas bottom shaded leaves have higher SLA (Ross and Ross 1996; Al Afas et al. 2007). Moderate changes in SLA can also be caused by nutrient and water stress whereas the impact of other possible factors is in general rather small (Poorter et al. 2009).

It has been demonstrated that there are less cytoplasmic compounds and more total structural carbohydrates and other cell wall compounds in leaves with low SLA, whereas leaves with higher water content have high SLA (Mediavilla et al. 2008; Poorter et al. 2009). Specific leaf area is lower in case of leaves with high leaf tissue density (e.g. Wilson et al. 1999), whereas leaf thickness in general does not affect SLA significantly (Poorter et al. 2009). In general it can be concluded that leaves with low SLA have smaller cells with thicker cell walls, packed together tightly.

In SRC willows with higher SLA are favoured because of their higher growth rate. However, plants with high SLA may become more vulnerable by unfavourable environmental conditions (Schulze et al. 2005) or pests. Leaves with low SLA are known to be more resistant to mechanical damages due to thicker cell walls and tend to be less preferred by insects due to smaller amount of sugars and water in the cells (Castro-Diez et al. 2000; Poorter et al. 2009). However, very little is known about relationships between plant pathogens and SLA. It could be speculated that lower SLA provides the plant with higher resistance to fungal pathogens such as rusts, since there is more lignin and cellulose in the cell walls. Thicker cell walls hinder the penetration by the fungi (Voegele and Mendgen 2003) and therefore should inhibit disease development and reduce susceptibility of the plant. On the other hand, differences in SLA values could also be changed by rust infection itself. In plant defence, sugars are transported to tissues and activate cell wall lignification at the infection site (Berger et al. 2007) at the same time the fungal biomass will increase. This could mean that more severely infected leaves could have smaller SLA since the infection could increase the mass per unit leaf area.

Previous studies have shown differences between willow clones and increasing rust levels in waste-water irrigated areas compared to controls (Toome et al. 2009). Since differences in rust severity were only partially described by shoot density inside the plantation (Toome et al. unpublished), a hypothesis was made that some differences in the leaf anatomy should be also responsible for observed leaf rust severity patterns. Therefore the aim of this study was to analyse the relation between SLA and leaf rust severity on leaves of different

SRC willow clones growing under various environmental conditions.

Materials and methods

The experiment was performed in Kambja SRC, established in 2003 in the southern part of Estonia. This plantation has three distinct parts, of which two were treated with pre-treated waste-water and one part was not irrigated. A detailed site description was given previously (Truu et al. 2009). In this study the areas with plant densities of 14 800 plants per ha were used. Sampling was conducted both in the control and in the waste-water-irrigated part with pipe distance of 4.5 m and irrigation holes at every 9 m. The average waste-water load in the treated part was 180 mm per vegetation season and with the concentrations of 16.5 and 2.7 mg/L for N and P, respectively. Three different willow clones were chosen for this study, based on their susceptibility to rust. Clones ‘78183’ and ‘78021’ belong to species *S. viminalis*; they are respectively susceptible and slightly susceptible to leaf rust; clone ‘81090’ belongs to species *S. dasyclados* and was also reported as susceptible in Estonia (Toome et al. 2009).

The leaves were sampled in the second part of September in 2005 and 2006 when the shoots were three and four years old, respectively. For sampling, five plants were randomly chosen from every clone/treatment, avoiding the border areas. From one shoot of each plant 15 full-sized leaves were randomly picked from the lower leaf horizon of the canopy. Leaves were dried at 65˚C for 48 h and the number of rust pustules on each leaf was counted. Thereafter the leaf was scanned and its area estimated using a computer program in order to estimate the number of pustules per unit leaf area. For estimating SLA, the leaves were dried again at 70˚C for 48 h and weighed immediately with a precision of 0.001 g. The SLA was calculated as a ratio between the leaf area and leaf dry weight. There were also uninfected leaves in most of the clone/treatment sets, which were irrelevant for the current study. The number of leaves in different data series used for the current study was unequal due to exclusion of uninfected leaves (Table 1).

In order to reduce heterogeneity in the residuals the number of rust pustules per leaf unit area was logarithmically transformed. Data analysis was conducted with SAS 8.02 (SAS Institute Inc., Cary, NC, USA). All differences were considered significant at $P<0.05$. Regression analysis was used to establish relationships between SLA and rust severity. Differences between the clones in rust severity and SLA were assessed with General Linear Models REGWQ test. To assess the differences between the clones and irrigation regimes, Least Squares Means test was used. For the latter analysis only data from year 2005 were used due to the very small sample size in the control in 2006 (Table 1).

Table 1 The number of infected leaves of studied three clones in control and waste-water-irrigated area in 2005 and 2006. The total number of sampled leaves was 900

	Control			Irrigated		
	‘78021’	‘78183’	‘81090’	‘78021’	‘78183’	‘81090’
2005	73	73	75	66	75	75
2006	7	0	2	62	75	75

Results

Specific leaf area was found to influence significantly the amount of leaf rust pustules on willow leaves ($P<0.0001$). There is strong evidence that higher SLA may be accompanied with higher rust levels on these leaves since there was a significant relationship between the average values of SLA and rust severity ($R^2=0.335$, $P<0.05$) (Fig. 1).

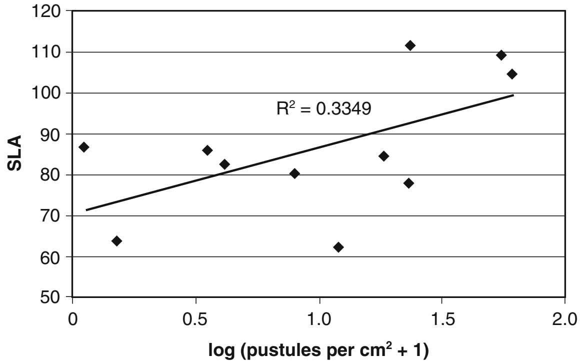

Fig. 1 Relation between willow leaf rust severity and specific leaf area (SLA). For this analysis the average values per willow clone, treatment and year obtained for both parameters were used

Table 2 Means and REGWQ grouping test results for specific leaf area (SLA) and rust severity (number of pustules per unit leaf area) of three willow clones

Clone	Mean SLA (cm^2/g)	REGWQ grouping[a]	Mean rust severity (log (pustules/cm^2+1))	REGWQ grouping[a]
'78183'	93.316	A	1.506	A
'81090'	93.180	B	1.172	B
'78021'	75.191	C	0.829	C

[a] Letters indicate the statistical difference between clones ($P<0.05$).

Pooled data from 2005 and 2006 showed that both SLA and rust severity differed significantly between willow clones ($P<0.0001$). SLA was the highest for the leaves of clone '78183', intermediate for '81090' and the lowest for clone '78021'. The same order was confirmed for the number of rust pustules (Table 2).

Waste-water irrigation increased significantly both SLA and rust severity, whereas control plants had significantly lower SLA and less rust damage ($P<0.0001$). When analysing the data from 2005 by clones, the waste-water irrigation increased SLA significantly only for clones '78183' and '81090' ($P<0.0001$). In the case of clone '78021', there was no difference between irrigated and control plants. The number of pustules increased significantly with waste-water irrigation for all studied clones ($P<0.0001$) (Fig. 2).

Discussion

The results of this study demonstrated a positive correlation between SLA and leaf rust severity. This correlation could be explained by leaf morphology. Since it is shown that leaves with low SLA contain more structural carbohydrates, such as lignin and cellulose in their cell walls (Mediavilla et al. 2008), it is possible that these leaves are not colonized by rust fungi as easily as leaves with thinner cell walls. The rust fungus produces special feeding structures, haustoria, for taking up nutrients from the plant. Haustoria form inside the plant cells and although the cell membrane is not penetrated, the fungus needs to break through the outer part of the cell wall (Voegele and Mendgen 2003), where most of the lignin and cellulose is located. Thus, if cell walls are thinner, *M. epitea* is able to form more haustoria to obtain more nutrients from the plant and hence to produce more rust spores. This would explain higher rust infections on leaves with higher SLA.

Another reason for low SLA associated with reduced rust development may be the existence of epicuticular waxes on some leaves. They consist mainly of long-chain fatty acids and alcohols with the main task to reduce water losses from the leaf epidermis and protect the tissue from chemical or biological attacks (Goodwin and Mercer 1986). In favourable conditions with no water stress the

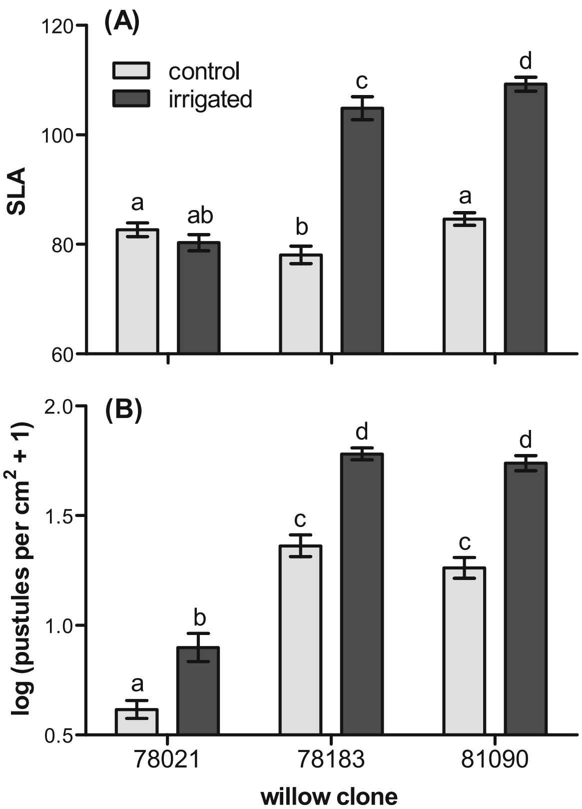

Fig. 2 Average specific leaf area (SLA) (**A**) and rust severity on leaves (**B**) of three different willow clones from control and wastewater irrigated area in 2005; error bars demonstrate standard errors. *Bars* with different letters indicate significant differences between the bars ($P<0.05$)

synthesis of these energetically-costly chemical compounds is diminished and therefore their effect of protecting the leaf from fungal attacks may become reduced. Leaves with low SLA could have thicker epicuticular wax layer and therefore be less susceptible to rust infections. This hypothesis is supported by a previous study (Pinon et al. 2006) where leaf wettability which is directly related to the wax layer on the leaves, was shown to be negatively correlated with successful rust infections.

A third reason for more rust on leaves with higher SLA could be the amount of carbohydrates, which are very important nutrients for obligate plant pathogens (Voegele and Mendgen 2003; Berger et al. 2007). It can be speculated that leaves with high SLA and thin cell walls contain more non-structural carbohydrates in their cell sap to keep up the turgor of large cells. In case of infection, these carbohydrates are easily available for rust and therefore will promote rust development. As well as carbohydrates, increased amounts of nitrogen and amino acids may also favour rust in leaves with high SLA (Voegele and Mendgen 2003). We could thus assume that leaves with high SLA are more favourable for rust fungi because of the increased availability of suitable nutrients. This agrees with our results that showed that leaves with more severe rust damage had higher SLA.

It has been shown previously that plant genotype influences significantly SLA values (e.g. Poorter et al. 2009). This study also found clear differences between willow clones. For all three clones, the ranking of clones for SLA values and rust severity were similar, with '78183' being the most susceptible and with the highest SLA and '78021' the least susceptible and with the lowest SLA.

The leaves of waste-water-irrigated plants were more severely infected by rust, which confirms previous results (Toome et al. 2009). Since it is known that more nutrients combined with optimal water availability change the leaf morphology, e.g. leaves become less dense and contain more nitrogen (Poorter et al. 2009), the result that the leaves from waste-water irrigated plants also have higher SLA was to be expected. Nevertheless, the response to waste-water treatment was clone specific since in case of clone '78021' the SLA of infected leaves was not significantly higher than for control. A similar tendency has been shown before for this clone for rust susceptibility (Toome et al. 2009). These results might suggest that stable SLA of clone '78021' under various environmental conditions indicates low adaptability. This may be one reason for less biomass production of this clone in favourable growing conditions than for other clones.

In conclusion, this study found that willow leaves with more rust infections also have higher SLA whether irrigated or not. Since these results are the first of their kind but are based on only three willow clones, additional studies are needed to determine if SLA and rust severity are positively correlated for other willow genotypes.

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References

Åhman, I. (1998). Rust scorings in a plantation of *Salix viminalis* clones during ten consecutive years. *European Journal of Forest Pathology, 28*, 251–258.

Åhman, I. & Wilson, F. (2008). Symptoms of pests, rust and other disorders on leaves of willow fertilized with wastewater, urine or sewage sludge. *Biomass and Bioenergy, 32*, 1001–1008.

Al Afas, N., Pellis, A., Niinemets, Ü. & Ceulemans, R. (2005). Growth and production of a short rotation coppice culture of poplar. II. Clonal and year-to-year differences in leaf and petiole characteristics and stand leaf area index. *Biomass and Bioenergy, 28*, 536–547.

Al Afas, N., Marron, N. & Ceulemans, R. (2007). Variability in Populus leaf anatomy and morphology in relation to canopy position, biomass production, and varietal taxon. *Annals of Forest Science, 64*, 521–532.

Begley, D., McCracken, A. R., Dawson, W. M. & Watson, S. (2009). Interaction in short rotation coppice willow, *Salix viminalis* genotype mixtures. *Biomass and Bioenergy, 33*, 163–173.

Berger, S., Sinha, A. K. & Roitsch, T. (2007). Plant physiology meets phytopathology: plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany, 58*, 4019–4026.

Castro-Diez, P., Puyravaud, J. P. & Cornelissen, J. H. C. (2000). Leaf structure and anatomy as related to leaf mass per area variation in seedlings of a wide range of woody plant species and types. *Oecologia, 124*, 476–486.

Coyle, D. R., Coleman, M. D., Durant, J. A. & Newman, L. A. (2006). Multiple factors affect pest and pathogen damage on 31 *Populus* clones in South Carolina. *Biomass and Bioenergy, 30*, 759–768.

Goodwin, T. W. & Mercer, E. I. (1986). *Introduction to plant biochemistry,* 2nd ed. Pergamon Press Ltd.

Heiska, S., Tikkanen, O.-P., Rousi, M., Turtola, S., Tirkkonen, V., Meier, B., et al. (2007). The susceptibility of herbal willow to *Melampsora* rust and herbivores. *European Journal of Plant Pathology, 118*, 275–285.

Johansson, L. K.-H. & Alström, S. (2000). Field resistance to willow leaf rust Melampsora epitea in inter- and intraspecific hybrids of Salix viminalis and S. dasyclados. *European Journal of Plant Pathology, 106*, 763–769.

Karp, A. & Shield, I. (2008). Bioenergy from plants and the sustainable yield challenge. *New Phytologist, 179*, 15–32.

Marron, N., Dillen, S. Y. & Ceulemans, R. (2007). Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. *Environmental and Experimental Botany, 61*, 103–116.

McCracken, A. R. & Dawson, M. (1992). Clonal response in Salix to Melampsora rusts in short rotation coppice plantations. *European Journal of Forest Pathology, 22*, 19–28.

McCracken, A. R. & Dawson, M. (1998). Short rotation coppice willow in Northern Ireland since 1973: development of the use of mixtures in the control of foliar rust (*Melampsora* spp.). *European Journal of Forest Pathology, 28*, 241–250.

Mediavilla, S., Garcia-Ciudad, A., Garcia-Criado, B. & Escudero, A. (2008). Testing the correlations between leaf life span and leaf structural reinforcement in 13 species of European Mediterranean woody plants. *Functional Ecology, 22*, 787–793.

Orians, C. M. & Floyd, T. (1997). The susceptibility of parental and hybrid willow to plant enemies under contrasting soil nutrient conditions. *Oecologia, 109*, 407–413.

Pinon, J., Frey, P. & Husson, C. (2006). Wettability of poplar leaves influences dew formation and infection by *Melampsora larici-populina*. *Plant Disease, 90*, 177–184.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J. & Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist, 182*, 565–588.

Ross, J. & Ross, V. (1996). Phytometrical characteristics of the willow plantation at Tõravere. In K. Perttu & A. Koppel (Eds.), *Short rotation willow coppice for renewable energy and improved environment* (pp. 133–145). Uppsala: Swedish University of Agricultural Sciences.

Royle, D. J. & Hubbes, M. (1992). Diseases and pests in energy crop plantations. *Biomass and Bioenergy, 2*, 45–54.

Schulze, E.-D., Beck, E. & Müller-Hohenstein, K. (2005). *Plant ecology*. Berlin/Heidelberg: Springer.

Toome, M., Heinsoo, K., Ramstedt, M. & Luik, A. (2009). Rust severity in bioenergy willow plantations treated with additional nutrients. *Forest Pathology, 39*, 28–34.

Truu, M., Truu, J. & Heinsoo, K. (2009). Changes in soil microbial community under willow coppice: the effect of irrigation with secondary-treated municipal wastewater. *Ecological engineering, 35*, 1011–1020.

Verwijst, T. (1993). Influence of the pathogen *Melampsora epitea* on intraspecific competition in a mixture of *Salix viminalis* clones. *Journal of Vegetation Science, 4*, 717–722.

Voegele, R. T. & Mendgen, K. (2003). Rust haustoria: nutrient uptake and beyond. *New Phytologist, 159*, 93–100.

Wilson, P. J., Thompson, K. & Hodgson, J. G. (1999). Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist, 143*, 155–162.