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FULL RESEARCH PAPER

The susceptibility of herbal willow to *Melampsora* rust and herbivores

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Abstract Eight Dark-leaved willow (*Salix myrsinifolia*) clones and two naturally hybridised clones (*S. myrsinifolia* × *S. phylicifolia*), that are considered to be suitable for herbal production, were cultivated for 2 years in Luikonlahti and Punkaharju. Both experimental sites are located in eastern Finland and the distance between the sites is 140 km. Different cultivation methods were used, including combinations of soil tillage, plastic mulch and fertilisation, with the aim of comparing the growth and susceptibility of plants to pathogens and willow-eating herbivores amongst the clones cultivated by different methods. In both study years 2001 and 2002, *Melampsora* rust-infected willows occurred in Luikonlahti and in Punkaharju. The extent of rust severity varied greatly between the years and experimental areas and amongst the clones. In 2002, fertilisation increased rust severity in Luikonlahti, but the effect was the converse in Punkaharju. Mulch effect on rust severity was clone-dependent. Vole feeding was observed in 56% of the plants in Luikonlahti during the winter 2001–2002 and the frequency of damaged plants was nearly twice as much amongst the willows grown in unmulched soil as those with plastic mulch. Cultivation method had no effect on feeding by leaf beetles or the abundance of aphids. Of the cultivation methods tested here, plastic mulch seems to have the most important influence on willow cultivation, particularly by improving willow growth and also by decreasing winter-feeding by voles.

Keywords Fertiliser · Cultivation reliability · Plastic mulch · *Salix*

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Introduction

A cultivation system for ‘herbal willow’, a willow that can be used as raw material for herbal drugs, has recently been studied with the aim of producing plant biomass that contains a high concentration of salicylates (Julkunen-Tiitto and Meier 1992; Heiska et al. 2005). Salicylates, a group of low molecular weight phenolic glucosides, are typically found in the bark and leaves of naturally grown willow species (Julkunen-Tiitto 1989). Since ancient times, willow bark has been used in folk medicine to relieve for example, headache and rheumatic pain (Pierpoint 1994). At present, salicylates can be extracted easily from harvested willow in the laboratory. The extract, made of willow bark, clearly shows analgesic and anti-inflammatory activity and is well tolerated in patients (Schmid et al. 2001a, b).

Northern dark-leaved willow (*Salix myrsinifolia*) can be considered for herbal willow cultivation because of its rapid growth and ability to produce a high concentration of salicylates (Meier et al. 1988; Julkunen-Tiitto and Meier 1992). In a cultivation system with a 2-year harvesting cycle, a reasonable yield of biomass and salicylates can be achieved by a cultivation technique that combines the use of plastic mulch and soil fertilisation. Plants with several branches instead of only a few are considered to be optimal for cultivation, because the proportion of salicylate containing bark is high in branches with a small diameter (Heiska et al. 2005). A cultivation method that promotes the growth, may however, impair the resistance of herbal willows to pathogens and several insect herbivores (Orians and Fritz 1996; Orians and Floyd 1997, but see Hakulinen 1998). Selecting plants with shrubby growth form for cultivation may also deteriorate cultivation reliability by assisting in the formation of a warm and humid microclimate in the foliage that favours the reproduction of fungal pathogens.

Of willow diseases, leaf rust is considered the most detrimental to plant growth (Abrahamson et al. 2002). Several species and pathotypes belonging to the genus *Melampsora* (Basidiomycota, Uredinales) infect cultivated willows leading to the outbreak of willow leaf rust, a fungal plant disease that can be seen as orange uredia in the leaves of infected willows (Pei et al. 1996, 1999; McCracken et al. 2000). The composition of the pathotypes within the pathogen population varies between geographic region and the pathotypes also vary widely in their host specificity. Willow infecting pathotypes are known to have *Larix* spp. and other conifers, *Ribes* spp. or *Allium* spp. as alternate hosts (Pei et al. 1993). A form of *Melampsora* rust without a known alternate host has also been found (Pei et al. 1995).

Heavy infection of leaf rust interferes with willow growth (Dawson and McCracken 1994; Abrahamson et al. 2002) and reduces winter hardiness (Verwijst 1990). Contamination of willow raw material, especially by fungal pathogens, may also lower yield quality by hindering the processing of the material. In addition to plant pathogens, there are a great number of insect herbivores feeding on willow leaves and stems during the growing seasons. In nature, herbivores may cause severe damage to willow vegetation (Sipura et al. 2002), but their effect on cultivated willows is considered to be of minor importance (Royle and Ostry 1995). Winter-feeding by voles, however, may cause considerable losses of biomass, especially when the voles are abundant (Gill 1992).

For improving yield quality and minimizing the risks in cultivation, the reliability of the cultivation methods should be appraised. Chemical pesticides cannot be recommended for herbal willow, because of the possibility of residues in the end product (Zuin and Vilegas 2000). Therefore, pest control must be based on cultivation technique and resistant clones. To elucidate the risks in cultivation, we examined the effects of soil mulching and fertilisation on the susceptibility of ten herbal willow clones to leaf rust and the distribution of willow eating herbivores in the field amongst the plants cultivated by different methods. We also studied the impact of these factors on willow growth and survival.

Materials and methods

Mulch and fertilisation experiment in Luikonlahti

Eight clones of dark-leaved willow and two naturally hybridised clones (*S. myrsinifolia* × *S. phylicifolia*, clones 9 and 10) were selected on the basis of their intense phenotypic growth and cultivated over two growing seasons (2001–2002) in an abandoned hay field in Luikonlahti (62°54 N; 28°40 E). The study site has a cool continental climate with a mean annual precipitation of about 600 mm and mean July temperature of 17.1°C. The average thermal growing season is 161 days. The soil type of the area is lacusterine gyttja with organic matter content of 39% and the subsoil below the plough layer is heavy clay.

Willows were planted from cuttings on ploughed and harrowed soil in late spring 2001 and cultivated by different methods for 2 years as described in Heiska et al. (2005). Cultivation methods consisted of all six combinations of two mulching treatments (black polythene mulch applied to 50% of the plants prior to planting and bare soil to 50%) and three fertilisation levels (control low and high fertiliser rates, each applied to one third of the plants within the mulching treatments). Plants grown in low fertiliser rate received 5–4–7 kg ha^{-1} N–P–K and 25–18–35 kg ha^{-1} N–P–K in 2001 and 2002 respectively, and those in high fertiliser rate received 20–14–38 kg ha^{-1} N–P–K and 150–100–283 kg ha^{-1} N–P–K in 2001 and 2002, respectively. Application was made with granular NPK-fertiliser (Puutarhan Y 1, Kemira Agro Ltd.). Control was unfertilised. Experimental design was a randomised complete split-plot block (Cochran and Cox 1957) with eight blocks, cultivation method (six levels) as the main-plot factor and clone (10 levels) as the sub-plot factor. Plot size was 1.0 × 1.3 m and each plot contained 12 cuttings, belonging to the same clone. In aggregate, there were 5,760 cuttings in the experimental area; 2,880 cuttings for each mulch treatment, 1,920 cuttings for each fertilisation treatment and 576 cuttings for each clone.

Fertilisation experiment in Punkaharju

During 2001–2002, the same clones cultivated in the Luikonlahti experiment were also used for a smaller scale fertilisation experiment in Punkaharju (61°48 N; 29°20 E, WGS-84), located 140 km south of Luikonlahti and having a climate comparable to that of Luikonlahti (mean annual precipitation of about 600 mm, mean July temperature of 17.1°C and the average thermal growing season of 162 days). Soil type of the area is sandy loam with an organic content of 11%. The study area was formerly growing shrubs and small sized trees, which were hacked down before the experiment was set up. Willow cuttings were planted on an untilled soil in late spring and the plants were cultivated for 2 years under different fertilisation levels as described in Heiska et al. (2005). Fertiliser rates were the same as in the Luikonlahti experiment. Experimental design was a randomised complete split-plot block (Cochran and Cox 1957) with five blocks, fertilisation (three levels) as the main-plot factor and clone (ten levels) as the sub-plot factor. Plot size and cutting density were the same as in Luikonlahti. The total amount of the cuttings in the experimental area was 1,800; 600 cuttings for each fertilisation treatment and 180 cuttings for each clone.

Rust and herbivore observations

For the analysis of natural *Melampsora* rust occurrence, four plants were chosen at random in each plot in the Luikonlahti and Punkaharju experiments. At the turn of August and September in 2001 and 2002, the youngest fully expanded leaf of each test plant was collected and dried under a weight in a drying-room at room temperature and RH of 10%. Dry leaves were stored in a freezer (−20°C). The severity of orange uredia on the lower surface of each leaf was observed from three pre-planned 5 × 5 mm^2 areas using a microscope with a 1 mm^2 grid. Each 1 mm^2 square occupied by orange pustules was counted so that each observed 25 mm^2 area was rated with a rust severity ranging from 0 to 25. Numbers were transformed to an average of rust severity in relation to leaf area (%).

The abundance of willow leaf and stem eating herbivores in the Luikonlahti and Punkaharju experimental areas was observed during both growing seasons. In early August 2001, when the number of leaf-eating herbivores was high in both experimental sites, six plants per each plot were selected for an estimation of the damage done by the most defoliating insects such as leaf beetles (*Phratora vitellinae* and *Galerucella lineola*) (Chrysomelidae, Coleoptera) and moth larvae (Noctuidae, Lepidoptera). The damage levels of plants were estimated from one typical shoot of the plant. The total number of leaves in these test shoots was counted and the proportion of damaged leaf area in the same shoots was visually rated at the accuracy of 0.5 leaves. Mean damage proportion of the plot was then calculated for statistical analysis.

The abundance of voles was high in Luikonlahti during the winter 2001–2002. In each plot, winter-damage was estimated by counting the total number of plants that showed any signs of vole feeding in spring 2002. Vole feeding was then calculated as the number of eaten plants in relation to the total number of plants (%). Vole feeding was not found in the Punkaharju experiment.

In the beginning of July 2002 when aphids (Aphididae, Homoptera) were observed as dense colonies in shoot top parts, three plants in each plot were systematically chosen for the estimation of aphid abundance in the Punkaharju experiment. The number of aphids in each shoot of the test plants was counted, except in dense colonies where only the number of aphids in a 1 cm piece of shoot tip was counted and the length of the rest of the colony was measured. Data were then transformed to number of aphids per plant and the frequency of infested plants. A high density of aphids was not observed in Luikonlahti, and thus not measured.

Some malformations in the tips of willow shoots, caused by European rosette willow gall midge (*Rhabdophaga rosaria* Cecidomyiidae, Diptera), were observed in the Punkaharju experiment during the summer of 2002. The number of malformations produced by the end of the growing season was counted on the same plants used for the analysis of rust occurrence.

Willow growth and survival

In both experimental sites, willow growth was measured on the same plants used for the analysis of rust occurrence. During the growing seasons, the length of their highest shoots from base to apex, hereafter called 'main shoot height', was measured in both experiments six times. In 2001, the growing season of the willows was considered to begin at the day of planting, June 5th and June 6th, in Luikonlahti and Punkaharju, respectively. Measurements during the growing season 2001 were made on July 16th and August 7th in Luikonlahti and on July 19th and August 8th in Punkaharju. The third measurement in 2001 was performed on August 11th and August 29th in Luikonlahti and Punkaharju, respectively, when the terminal buds were formed and the growing season of the willows terminated. In 2002, the growing season began on May 8th, at the time of bud burst. Measurements during the growing season were made on July 1st and August 2nd in Luikonlahti and on June 26th and August 5th in Punkaharju. The final main shoot height in both experiments was measured on September 2nd, when the terminal buds were formed.

Growth curves representing the change in main shoot height during the growing seasons were drawn on *x*–*y* co-ordinates with observed main shoot height on the *y*-axis and number of days of the growing season on the *x*-axis. The area under the curves, hereafter called 'total growth', was calculated.

Shoot numbers of the same plants used for total growth measurements were also measured at the end of both growing seasons, as described by Heiska et al. (2005). The numbers of all living and dead plants were counted in autumn 2001 and spring and autumn 2002. Winter survival (%) of the plants was calculated as the number of living plants in spring 2002 and compared to those counted in autumn 2001. Mortality was expressed as the proportion (%) of dead plants compared to the number of plants originally planted in spring 2001.

Statistical analysis

An analysis of variance (ANOVA) with a model for split-plot design was used to test the differences in rust occurrence. A linear mixed model ANOVA for repeated measures was used to analyse the differences in willow main shoot height. A linear mixed model ANOVA with split-plot design was computed for the total growth, vole feeding, willow shoot number, mortality and winter survival. The variation in willow mortality between the clones was tested using Bonferroni-adjusted pairwise comparisons of estimated marginal means. The effects of clone and cultivation method on leaf damage caused by herbivores were tested with separate Friedmans χ^2 tests. Multiple Bonferroni adjusted pairwise comparisons for the difference in leaf damage between the clones were made using Wilcoxon's signed rank test. Cochran's Q was used to test the effect of clone on the frequency of the plants infested by aphids. The applicability of the statistical test was examined from residual plots and the homogeneity of variances was tested with Levene statistics (critical $\alpha = 0.05$). Due to non-homogeneity of the variances of the rust data, an angular transformation of observed values was used for parametric tests. Results of the rust data are presented as observed values. SPSS 10.1 for Windows was used for all statistical analysis.

Results

Willow foliar rust and herbivore feeding

In 2001, first uredia were observed on willow leaves in late-July, whilst in 2002 uredia were visible as early as mid-June. Across all clones and treatments in Luikonlahti, rust severity in late summer was 6% in 2001 and 21% in 2002 (Fig. 1a, b). The average rust severity in Punkaharju was 6% in 2001 and 32% in 2002 (Fig. 2a, b). Willow susceptibility to rust was highly clone-dependent in both years and in both experimental sites (Figs. 1, 2). In 2001, clone 8 was overwhelmingly the most susceptible to rust in both sites and in 2002, clones 2, 8 and 10 were the most infected (Figs. 1, 2).

Fertilisation did not have any effects on rust severity in 2001, whilst in 2002, the severity was increased by 25% amongst the fertilised plants compared to unfertilised control plants in Luikonlahti (Fig. 1). In Punkaharju, fertiliser effect was the opposite, but the decrease in rust severity of fertilised plants was small compared to the unfertilised control (Fig. 2). Mulch did not have any effects on rust severity in 2001; however, in 2002, a significant mulch-by-clone interaction indicated that the effect of mulch varied amongst the clones (Fig. 1). The effect of mulch was especially high in clone 6 (Fig. 1b).

Damage caused by leaf-eating herbivores in 2001 was observed in 49 and 87% of the plants in Luikonlahti and Punkaharju respectively. An average of 2% of the leaves of plants grown in Luikonlahti and 10% of those in Punkaharju were eaten by herbivores (Fig. 3a, b). Based on the feeding pattern, the damage was mainly caused by leaf beetles. Herbivores preferred clones 1 and 9 in both experimental sites (Fig. 3a, b).

Voles damaged 56% of the plants in Luikonlahti during the winter 2001–2002 (Fig. 4) Vole feeding was highly affected by clone and even more by plastic mulch. The frequency of willows eaten by voles in bare soil was nearly two-fold that in plastic mulch. The effect of mulch was, however, not of the

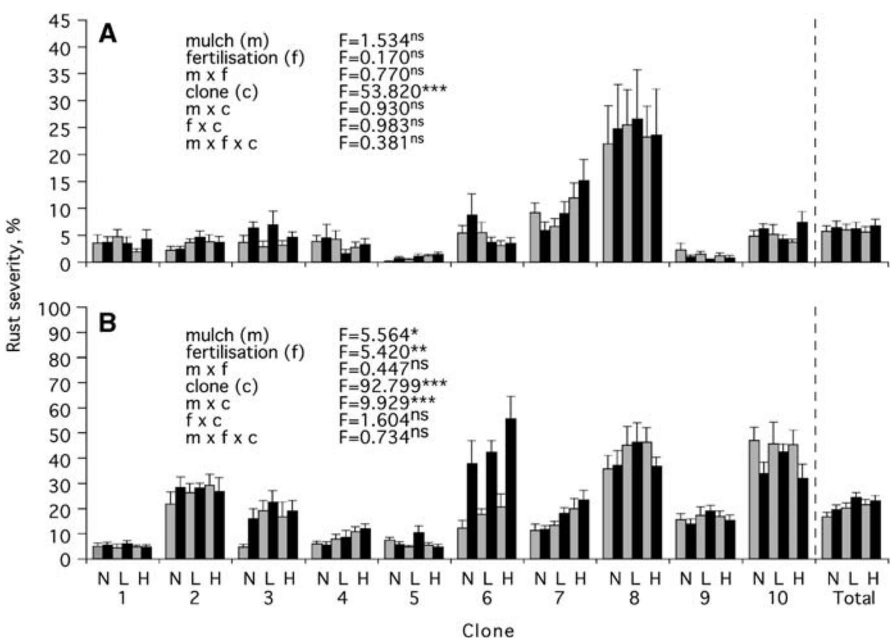

Fig. 1 Effect of cultivation method and clone on *Melampsora* rust severity in Luikonlahti, 2001 (**a**) and 2002 (**b**). Grey and black bars represent observed means of willows grown in bare soil and with plastic mulch, respectively. Error bars indicate SE of the means. Letters N, L and H below the bars indicate none, low, and high levels of fertilisation. *F*-values for the experimental factors and their interactions are presented; asterisks, *, ** and *** indicate levels of significance, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Ns: not significant. Statistical tests were made on angle-transformed data, the original data are presented here. Note the different scales on the *Y*-axis

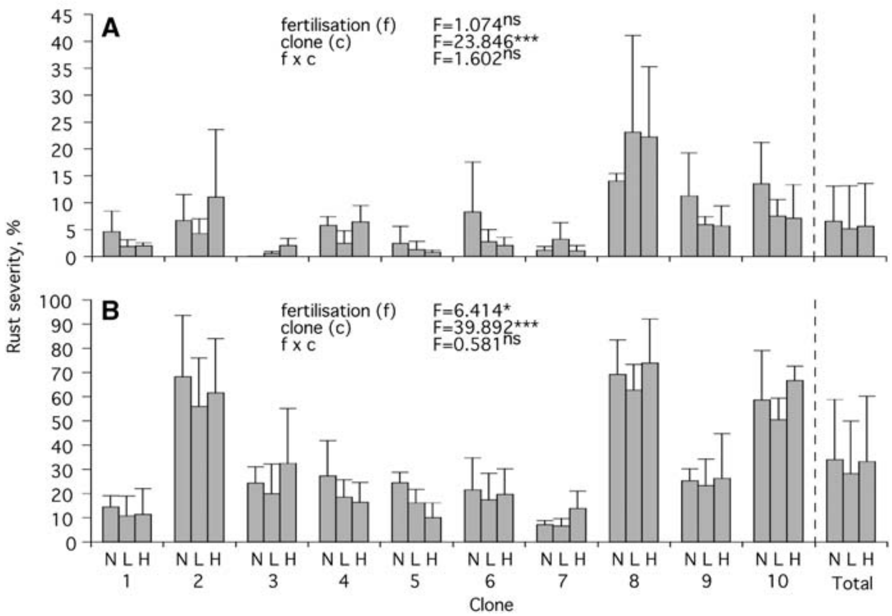

Fig. 2 Effect of cultivation method and clone on *Melampsora* rust severity in Punkaharju, 2001 (**a**) and 2002 (**b**). Bars represent means and error bars indicate SE for the means. Letters N, L and H indicate none, low, and high levels of fertilisation, respectively. *F*-values for the experimental factors and their interactions are presented; asterisks, *, ** and *** indicate levels of significance, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Ns: not significant. Statistical tests were made in angle-transformed data, the original data are presented here. Note the different scales on the *Y*-axis

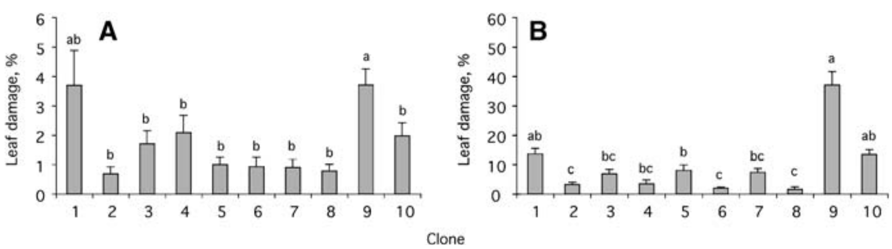

Fig. 3 Leaf damage by insect herbivores in Luikonlahti (**a**) and in Punkaharju (**b**), 2001. Bars represent averages of the clones and error bars indicate SE. Mean values of the clones marked with same letters do not differ from each other based on multiple pair-wise comparisons by Bonferroni adjusted Wilcoxon signed rank test, $P < 0.05$

same magnitude in all the clones resulting in a weak mulch by clone interaction. In Punkaharju, voles did not damage the experimental willows during the study years.

Aphids were observed in 12% of the plants in Punkaharju. In most of the infested plants there were <50 aphids; >1,000 aphids were observed on only one plant. The frequency of infested plants was clearly dependent on clone (Cochrans Q, $P < 0.01$). Malformations caused by *Rhabdophaga* gall midges were found in only 3% of the plants in Punkaharju and no difference amongst the clones could be seen.

Willow growth and survival

The average main shoot height after 2 years was 126 (±1.2, SE) cm in Luikonlahti and 51 (1.1 ±SE) cm in Punkaharju. In both experiments, the main shoot height was highly dependent on the clone (Table 1). Fertilisation increased main shoot height in both experiments, but the fertiliser effect was not significant until the second growing season (Table 1). Plant responses to fertilisation were clone-specific shortly after the first fertiliser application in Punkaharju, but subsequent fertiliser-by-clone interaction was

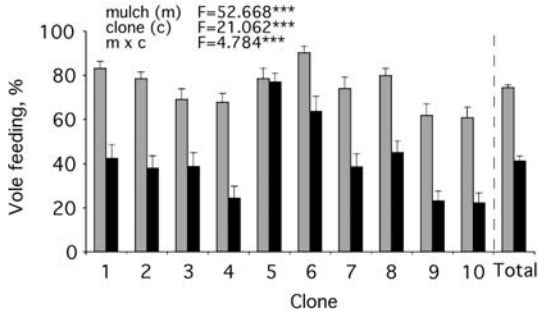

Fig. 4 Vole feeding in Luikonlahti during winter 2001–2002 expressed as the number of plants browsed by voles in relation to the total number of plants (%). Grey and black bars represent means of willows grown in bare soil and plastic mulch, respectively. Error bars indicate SE for the means. F-values for the experimental factors and their interactions are presented; asterisks, *, ** and *** indicate levels of significance, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Only significant effects and their interactions are presented in the figure

insignificant (Table 1). Fertilisation increased the average final main shoot height by 12 and 36% compared to the unfertilised control in Luikonlahti and Punkaharju, respectively. In Luikonlahti, plastic mulch considerably increased plant growth and this was evident from the very first growth measurements. The magnitude of the mulch effect varied amongst the clones and during the whole cultivation period; however, the mulch-by-clone interaction for main shoot height disappeared by the end of the 2-year cultivation period (Table 1). Plastic mulch increased the average final main shoot height by 36% compared to the plants grown in bare soil.

Willow total growth, measured as area under the growth curves for shoot height over both seasons was 131 and 44 in Punkaharju and Luikonlahti respectively (Fig. 5a, b). In both experiments, the total growth was highly dependent on clone. Fertilisation increased the total growth of willows compared to that in bare soil in Punkaharju (Fig. 5a). In Luikonlahti, plastic mulch clearly increased the total growth compared to willows grown in bare soil, but the magnitude of the increase was clone-dependent (Fig. 5b).

The average shoot numbers at the end of the growing season of 2001 were 3.1 (±1.0, SD) and 2.7 (±1.0, SD) in Luikonlahti and Punkaharju respectively. In 2002, the corresponding values were 2.8 (±1.0, SD) in Luikonlahti and 2.3 (±1.5, SD) in Punkaharju (Heiska et al. 2005). In both locations, shoot number was highly determined by clone (ANOVA, $P < 0.001$ for clone effect) with clones 1, 8 and 10 having the highest numbers of shoots. In Luikonlahti, shoot number of the plants grown in plastic mulch was increased by 9 and 21% compared

Table 1 Results of the repeated measures mixed-model analysis of variance for willow height increments during 2 years of cultivation in Luikonlahti and Punkaharju experiments, 2001–2002: summary of P-values (P-values less than 0.05 are italised)

Source of variation	2001			2002		
	Jul	Aug	Sep	Jul	Aug	Sep
Luikonlahti						
Intercept	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Mulch (M)	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Fertilization (F)	0.883	0.930	0.934	0.421	*0.003*	*0.001*
M*F	0.610	0.642	0.916	0.335	0.508	0.342
Clone (C)	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
M*C	*0.000*	*0.001*	*0.008*	*0.001*	*0.013*	0.051
F*C	0.646	0.394	0.134	0.345	0.379	0.182
M*F*C	0.578	0.403	0.348	0.356	0.765	0.798
Punkaharju						
Intercept	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
Fertilization (F)	0.702	0.288	0.098	*0.028*	*0.000*	*0.000*
Clone (C)	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*
F*C	*0.000*	0.610	0.667	0.051	0.083	0.053

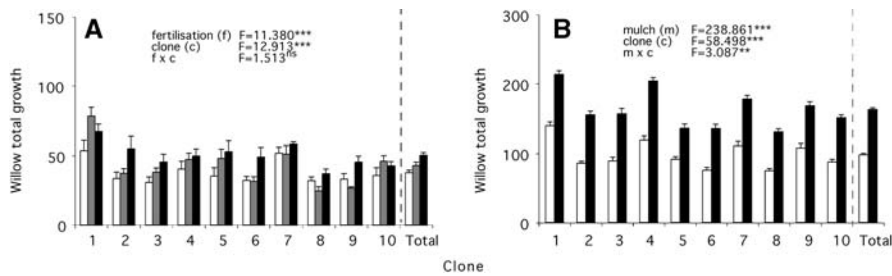

Fig. 5 Willow total growth expressed as area under the curve in Punkaharju (**a**) and Luikonlahti (**b**). In (**a**) white, grey and black bars indicate the mean values of willows cultivated at none, low and high levels of fertilisation, respectively and in (**b**) white and black bars indicate the mean values of the willows grown in bare soil and plastic mulch, respectively. Error bars indicate SE for the means. *F*-values for the experimental factors and their interactions are presented; asterisks, *, ** and *** indicate the levels of significance, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Only significant effects and their interactions are presented in the figure. Note the different scales on the *Y*-axis

to those in bare soil in 2001 and 2002 respectively (ANOVA, $P < 0.01$ and $P < 0.001$, for mulch effect). In 2002, mulch effect was, however, dependent on clone (ANOVA, $P < 0.01$, for mulch-by-clone interaction).

Mortality was greatly affected by clone (ANOVA, $P < 0.001$) and clone 5 had the highest values in both experimental sites. Winter survival of the plants was good; an average of 6 and 12% of the plants in Luikonlahti and Punkaharju died during the winter (Fig. 6a, b). Overall, survival was highly clone-dependent in both experimental sites (ANOVA, $P < 0.001$), averaging 9 and 21% in Luikonlahti and Punkaharju, respectively, during the 2-year cultivation period.

Discussion

Rust severity was highly influenced by year and experimental site (Figs. 1, 2). The precipitation in eastern Finland was higher during summer (June–August) 2002 than in 2001, though in both years the precipitation was near the 30 year average.

Temperatures in July and August 2002 were exceptionally high. Differences in the climate between the years may have affected the reproduction of the pathogen and the abundance of willow-infecting spores, leading to different levels of rust severity between the years. Though *Melampsora* pathotype structure is known to vary depending on the site and vicinity of host plants, and pathotypes differ in their pathogenicity and host specifity (Ramstedt 1999; Niemi et al. 2006), the ranking order of the clones in our study seemed to be quite similar throughout the years and experimental sites. This suggests that that the *Melampsora* pathotype structure probably is quite similar in both experimental sites. The effect of cultivation method on rust occurrence was small, but results from Luikonlahti in 2002 showed that the use of fertilisation and plastic mulch can increase rust

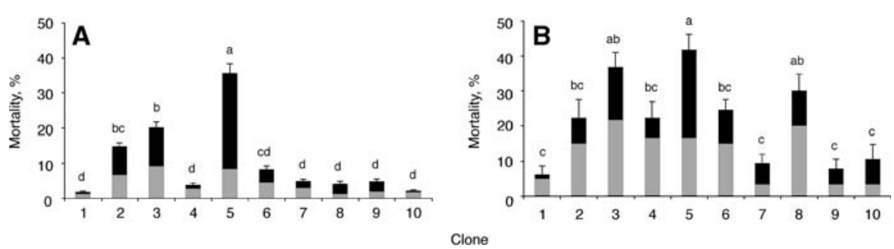

Fig. 6 Willow mortality in Luikonlahti (**a**) and Punkaharju (**b**) expressed as the proportion of dead plants at the end the growing season 2002 compared to the total number of plants planted in 2001. Bars represent mean values and error bars indicate SE for the means. Bars marked with different letters differ from each others based on Bonferroni adjusted multiple pair-wise comparisons of estimated marginal means ($P < 0.05$). Grey parts of bars indicate the amount of the plants that died during the winter 2001–2002

occurrence in some clones (Fig. 1b, see especially the effect of mulch in clone 6).

In energy plantations, heavy rust infection has been reported to reduce willow growth by, for example, accelerating leaf abscission (Dawson and McCracken 1994; Steenackers et al. 1996; Abrahamson et al. 2002). Infection can also interfere with plant growth in the following growing season by delaying bud burst (Steenackers et al. 1996) and reducing winter hardiness (Verwijst 1990). In our study, the total growth of willows was the highest in clones 1 and 4 in Luikonlahti and in clones 1 and 7 in Punkaharju (Fig. 5). In both experiments, the severity of *Melampsora* rust was low in these clones (Figs. 1, 2). In contrast, total growth was lowest in the most infected clone 8 (Fig. 5). However, differences in main shoot heights were seen from the first height measurement, before the emergence of the first uredia in willow leaves (Table 1). Therefore, we consider the effect of rust infection on shoot height to be of minor importance. In areas where summer precipitation is higher, the effect of rust on willow survival or growth is probably much higher (e.g. Dawson and McCracken 1994).

Air circulation in dense willow stands can be slow, producing particularly in rainy growing seasons, a warm and humid microclimate in the foliage that favours the reproduction of the pathogen. In our study, shoot number was higher in willows grown with plastic mulch than in those grown in bare soil. Increased canopy density may partly explain the strong effect of plastic mulch on rust occurrence in clone 6 (Fig. 1b). The high levels of rust infection in clones 8 and 10 were probably a consequence of their high shoot number (Heiska et al. 2005). On the other hand, although willow growth was slow in untilled soil in Punkaharju and the canopy stayed open during the whole experiment, rust severity in this site was again higher in 2002 (Fig. 2b) Therefore, we consider shoot number to have only a minor effect on rust severity. Heavy rust infection has been reported to undermine willow winter hardiness (Verwijst 1990). The occurrence of foliar rust was, however, quite low in 2001 and so there was no clear trend between rust occurrence and winter survival in this study (Figs. 1, 2 and 6).

In small trees, vole feeding can cause large losses of biomass, especially when the voles are abundant (Gill 1992). However, in most of the browsed willows in our study, bark was only partially removed and none of the plants were totally destroyed by voles. In Luikonlahti, the frequency of feeding damage made by voles was clearly decreased by the use of plastic mulch (Fig. 4). Plastic mulch greatly increased willow total growth (Fig. 5), probably by suppressing weed competition, levelling the variation in soil temperature and improving the water relations, and we found no relationship between the total growth and vole feeding within the mulch treatments. This indicates that vole feeding in our study did not, on the whole, affect final plant height. Also, no effects of vole feeding on willow winter survival or mortality during the experiment were found in our study.

In Punkaharju, the extent of damage made by beetles and other leaf-eating insect herbivores was nearly 10-fold that in Luikonlahti (Fig. 3). The difference between the experimental sites could partly be explained by the soil management practices prior to planting. The ploughing and harrowing of the experimental area in Luikonlahti probably disturbed the over-wintering of the beetles, leading to fewer leaf-eating herbivores observed later in the growing season. On the other hand, the number of beetles in the Luikonlahti experimental area probably did not increase rapidly as a response to the intense willow growth and increased amount of food. In consequence, the effect of beetle feeding, expressed as % leaf area, was obscured by willow growth. Nutrients and soil water relations have been shown to affect the susceptibility of willows and other broad-leaved trees to leaf beetles (Orians and Floyd 1997; Gruppe et al. 1999; Lower and Orians 2003). In our study, mulch and fertilization treatments had no effect on the feeding of leaf-eating herbivores (Friedman test, $P > 0.05$ for both experiments; Fig. 3a, b). Differences in beetle feeding amongst the clones, however, support the earlier findings that willow susceptibility to leaf beetles is strongly controlled by genetics (Hodkinson et al. 1998; Glynn et al. 2004). There was no clear relationship between growth and insect feeding at both sites.

We consider the difference in plant growth between the experimental areas to be mainly a consequence of the different soil management practices and of their effect on the early development of the cuttings. The cultivated area in Luikonlahti was badly infested by propagules of perennial weeds, mostly *Ranunculus repens*, which emerged within a few weeks after planting. In Punkaharju, the soil was not tilled and the willows were competing with weeds, mostly perennial grasses, starting at the day of planting. The difference in willow growth between the experiments may therefore be influenced by their weedless periods of unequal length and the different weed flora.

Plant stress is suggested to influence aphid abundance in birch, but plant vigour may also be an important factor. Johnson et al. (2003) reported aphids to be more abundant on vigorously growing birch branches than on those destined to become stressed; branch vigour influenced aphid distribution at the beginning of the growing season, but later in the season the role of stress became more important. In our study, the frequency of plants infested by aphids was highest in clones 1, 4, and 7, that also had the longest main shoots at the time of aphid observations (data not shown). Altogether, the effect of aphid infestation on willow growth and survival was considered to be minor in our study.

In the natural environment, willows often cope with a diverse variety of willow-attacking herbivores and pathogens. The system regulating pest abundance may be multi-trophic and complicated. Willow susceptibility to rust infection is known to increase as a consequence of feeding by leaf beetles, but on the other hand, infection deteriorates leaf quality, affecting herbivore performance and larval development (Simon and Hilker 2003). In our study with cultivated willow, no relationship between leaf eating herbivores and rust occurrence was found. Instead, winter-feeding by voles may have decreased willow susceptibility to *Melampsora* rust by reducing shoot number and improving air circulation within the willow stand. Heavier browsing during the peak years of the vole-cycle may, however, induce willow compensatory growth and lead to the formation of shrubby vegetation. Therefore, the influence of vole feeding on willow rust probably varies depending on vole abundance and the extent of feeding damage.

This study highlights the importance of selecting reliable clones suitable for a cultivation system. More long-term and multi-location experiments using the same conditions of soil tillage are, however, needed to prove the long-time reliability of herbal willow, pressured by the naturally fluctuating populations of pathogens and herbivores. Of the cultivation methods tested here, covering soil with plastic mulch seems to have the most important influence on the reliability of herbal willow cultivation, particularly by improving willow growth and salicylate yield (Heiska et al. 2005) and also by decreasing winter feeding by voles. More studies are needed, however, to show whether the explanation for vole feeding behaviour is found in willow growth and chemistry or in physical aspects of the plastic mulch itself.

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References

Abrahamson, L., Volk, T., Kopp, T., White, E., & Ballard, J. (2002). *Willow biomass producers handbook*. NY, USA: SUNY, 31 pp.

Cochran, W., & Cox, G. (1957). Factorial experiments with main effects confounded: Split-plot designs. In W. Cochran & G. Cox (Eds.), *Experimental designs* (2nd ed., pp. 293–316). NY, USA: John Wiley & Sons.

Dawson, W., & McCracken, A. (1994). The effects of *Melampsora* rusts on the growth and development of *Salix burjatica* Korso in N. Ireland. *European Journal of Plant Pathology, 24*, 32–39.

Gill, R. (1992). A review of damage by mammals in north temperate forests. 2. Small mammals. *Forestry, 65*, 281–308.

Glynn, C., Rönnberg-Wästljung, A.-C., Julkunen-Tiitto, R., & Weih, M. (2004). Willow genotype but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomologia Experimentalis et Applicata, 113*, 1–14.

Gruppe, A., Fusseder, R., & Schopf, R. (1999). Short rotation plantations of aspen and balsam poplar on former arable land in Germany: Defoliating insects and leaf constituents. *Forest Ecology and Management, 121*, 113–122.

Hakulinen, J. (1998). Nitrogen-induced reduction in leaf phenolic level is not accompanied by increased rust frequency in a compatible willow (*Salix myrsinifolia*)-*Melampsora* rust interaction. *Physiologia Plantarum, 102*, 101–110.

Heiska, S., Rousi, M., Turtola, S., Meier, B., Tirkkonen, V., & Julkunen-Tiitto, R. (2005). The effect of genotype and cultivation method on the total salicylate yield of dark-leaved willows (*Salix myrsinifolia*). *Planta Medica, 71*, 1134–1139.

Hodkinson, I., Flynn, D., & Shackel, S. (1998). Relative susceptibility of *Salix* clones to chrysomelid beetles: Evidence from the Stott willow collection at Ness. *European Journal of Forest Pathology, 28*, 271–279.

Johnson, S., Elston, D., & Hartley, S. (2003). Influence of host plant heterogeneity on the distribution of a birch aphid. *Ecological Entomology, 28*, 533–541.

Julkunen-Tiitto, R. (1989). Phenolic constituents of *Salix*: A chemotaxonomic survey of further Finnish species. *Phytochemistry, 28*, 2115–2125.

Julkunen-Tiitto, R., & Meier, B. (1992). Variation in growth and secondary phenolics among field-cultivated clones of *Salix myrsinifolia*. *Planta Medica, 58*, 77–80.

Lower, S., & Orians, C. (2003). Soil nutrients and water availability interact to influence willow growth and chemistry but not leaf beetle performance. *Entomologia Experimentalis et Applicata, 107*, 69–79.

McCracken, A., Dawson, W., Watson, S., & Allen, C. (2000). Pathotype composition in *Melampsora epitea* populations occurring on willow (*Salix*) grown in mixed and monoculture plantations. *European Journal of Plant Pathology, 106*, 879–886.

Meier, B., Sticher, O., & Julkunen-Tiitto, R. (1988). Pharmaceutical aspects of the use of willows in herbal remedies. *Planta Medica, 54*, 559–560.

Niemi, L., Wennström, A., Hjältén, J., Waldmann, P., & Ericson, L. (2006). Spatial variation in resistance and virulence in the host-pathogen system *Salix triandra–Melampsora amygdalinae*. *Journal of Ecology, 94*, 915–921.

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

Orians, C., & Fritz, R. (1996). Genetic and soil-nutrient effects on the abundance of herbivores on willow. *Oecologia, 105*, 388–396.

Pei, M., Hunter, T., & Ruiz, C. (1999). Occurrence of *Melampsora* rusts in biomass willow plantations for renewable energy in the United Kingdom. *Biomass and Bioenergy, 17*, 153–163.

Pei, M., Royle, D., & Hunter, T. (1993). Identity and host alternation of some willow rusts (*Mealampsora* spp.) in England. *Mycological research, 97*, 845–851.

Pei, M., Royle, D., & Hunter, T. (1995). A comparative study of stem- and leaf-infecting forms of *Melampsora* rust on *S. viminalis* in the U.K. *Mycological Research, 99*, 357–363.

Pei, M., Royle, D., & Hunter, T. (1996). Pathogenic specialization in *Melampsora epitea* var. *epitea* on *Salix*. *Plant Pathology, 45*, 679–690.

Pierpoint, W. S. (1994). Salicylic acid and its derivatives in plants, medicines, metabolites, and messenger molecules. *Advances in Botanical Research, 20*, 163–235.

Ramstedt, M. (1999). Rust disease on willows—Virulence variation and resistance breeding strategies. *Forest Ecology and Management, 121*, 101–111.

Royle, D., & Ostry, M. (1995). Disease and pest control in the bioenergy crops poplar and willow. *Biomass and Bioenergy, 9*, 69–79.

Schmid, B., Kötter, I., & Heide, L. (2001a). Pharmacocinetics of salicin after oral administration of a standardized willow bark extract. *European Journal of Clinical Pharmacology, 57*, 387–391.

Schmid, B., Lüdtke, R., Selbmann, H.-K., Kötter, I., Tschirdewahn, B., Schaffner, W., & Heide, L. (2001b). Efficacy and Tolerability of a standardized willow bark extract in patients with osteoarthritis: Randomised placebo-controlled, double-blind clinical trial. *Phytotherapy Research, 15*, 344–350.

Simon, M., & Hilker, M. (2003) Herbivores and pathogens on willow: Do they affect each other? *Agricultural and Forest Entomology, 5*, 275–284.

Sipura, M., Ikonen, A., Tahvanainen, J., & Roininen, H. (2002). Why does the leaf beetle *Galerucella lineola* F. attack wetland willows? *Ecology, 83*, 3393–3407.

Steenackers, J., Steenackers, M., Steenackers, V., & Stevens, M. (1996). Poplar diseases, consequences on growth and wood quality. *Biomass and Bioenergy, 10*, 267–274.

Verwijst, T. (1990). Clonal differences in the structure of a mixed stand of *Salix viminalis* in response to *Melampsora* and frost. *Canadian Journal of Forest Research, 20*, 602–605.

Zuin, V., & Vilegas, J. (2000). Pesticide residues in medicinal plants and phytomedicines. *Phytotherapy Research, 14*, 73–88.