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Site-specific field resistance of grapevine to *Plasmopara viticola* correlates to altered gene expression and was not modulated by the application of organic amendments

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Abstract The influence of site on resistance of grapevine (*cv.* Chasselas) to *Plasmopara viticola* was evaluated. Grapevine leaves from three vineyards in the region of Lake Neuchâtel (Switzerland) were tested for their susceptibility to *P. viticola* in the lab in five successive years (2004–2008), and the expression levels of four selected defence-related genes (*Glucanase*, *Lipoxygenase 9*, *9-cis epoxycarotenoid dioxygenase*, *Stilbene synthase*) were studied in 1 year. In all 5 years of examination, differences between sites were substantial. In four out of 5 years, plants from site Hauvernier were much less susceptible to *P. viticola* than plants from site Auvernier. In another year, differences were less pronounced but still significant for one leaf age. Susceptibility of plants from a third site (Concise) varied from year to year. Differences in the genetic background were excluded by microsatellite analysis. Differences in susceptibility were mirrored in the constitutive expression pattern of four defence-related genes, with samples from the Hauterive site clearly separated from samples of the other two sites in redundancy analysis. Furthermore, it was evaluated whether site-specific resistance can be modulated by agronomic practices such as the application of organic amendments. In two commercial vineyards (*cv.* Pinot noir), soils had either not (control) or yearly (compost) been amended with a compost for the last 9 years. Leaves from plants grown in any of the two treatments did not differ in their susceptibility to *P. viticola* in both years of examination. Additionally, under controlled conditions, none of 19 different composts amended to the substrate of grapevine seedlings or cuttings affected their susceptibility to *P. viticola*, but 8 out of 19 composts reduced severity in the control bioassay *Arabidopsis thaliana—Hyaloperonospora arabidopsidis*, indicating that a modulation of site-specific susceptibility of grapevine plants by organic amendments is at the very least, difficult.

Keywords Compost · Induced systemic resistance · Soil · Terroir · *Vitis vinifera*

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Introduction

Site has long been recognized as being an important factor in influencing plant well-being. Macro- and micro-climate, soil structure, soil nutrient content and the soil microbial population influence the well-being of plants directly but also indirectly by affecting

occurrence and infectious potential of plant pathogens. Soils in which plants develop no or reduced disease symptoms, even if a pathogen is present or artificially added to the soil, are known as suppressive soils (Weller et al. 2002). Certain soils even influence the susceptibility of plants to foliar diseases (Tamm et al. 2010). Living soil microorganisms have been identified as a key factor to reduce the occurrence and severity of soil-borne diseases (Knudsen et al. 2002; Menzies 1959; Shipton et al. 1973; Stutz et al. 1986; Wiseman et al. 1996), as well as the resistance of plants to foliar diseases (Thuerig et al. 2009). Soil microorganisms can control soil-borne pathogens through competition, antibiosis, hyperparasitism and the induction of plant disease resistance (Haas and Défago 2005). In the case of foliar diseases, beneficial soil microorganisms and pathogens are spatially separated and the soil microorganism-induced defence-state of the plant is referred to as induced systemic resistance (ISR) (van Loon et al. 1998). Various root associated bacteria, mainly fluorescent pseudomonads, that enhance resistance of various plants to several foliar diseases have been identified (van Loon et al. 1998). In general, ISR is not associated with major changes in the constitutive expression of defence genes, but with a faster and/or stronger activation of basal defence mechanisms such as the expression of defence-related genes upon contact with a pathogen, also referred to as priming (Conrath et al. 2006). The occurrence of ISR against foliar diseases has been demonstrated in experiments under controlled conditions in annual plants mainly, while under field conditions and in woody perennial plants such as grapevine, only little is known about occurrence and relevance of this phenomenon (Kloepper et al. 1999). Yet, it has been shown that *in vitro* cultured grapevine plantlets can be colonised by beneficial pseudomonads, promoting growth of the plants and their resistance to *Botrytis cinerea* (Ait Barka et al. 2000), and that colonization of grapevine roots with a mycorrhizal fungus induced defence-responses against root-knot nematodes, including transcription of a chitinase (Li et al. 2006). Furthermore, the concept of 'terroir' is well known in viticulture and implies that there is a strong relationship between the composition of the grape, the characteristics of the wine and the territory of production (Morlat and Bodin 2006; Seguin 1986). Besides climate, plant material and anthropogenic influence, soil has been shown to be a main determinant of a defined 'terroir' and influences grape quality parameters such as sugar, anthocyanin, and phenolic contents (Downey et al. 2006; Jackson and Lombard 1993; Morlat and Bodin 2006). Many geological and pedological factors have been shown to have an important impact on vine and grape quality. Recently, Morlat and Bodin (2006) suggested that the type and weathering of parental rock are key factors for the grape quality within a certain climatic area, by affecting parameters such as mineral nutrient content, soil depth, clay content and water storing capacity.

For commercial agriculture, it is of crucial importance whether site-specific suppressiveness can be influenced by appropriate management techniques. Several studies have demonstrated that suppressiveness of natural soils to soil-borne diseases can be modulated by the application of organic soil amendments such as manure and compost (Fuchs et al. 2004; Litterick et al. 2004), and a few reports have shown the same for suppressiveness of soils to air-borne diseases (Tamm et al. 2010; Vallad and Goodman 2004). Yet, differences between management strategies were much smaller than differences between soil types (Tamm et al. 2010), and little is known about the effect of soil management practices on the resistance of woody perennial plants to foliar air-borne diseases.

The main objective of this study was to investigate the impact of site and organic soil amendments on the resistance of grapevine to *Plasmopara viticola*. We investigated whether i) grapevine plants of the same variety growing at different sites in the same region differ in their resistance to *P. viticola*, ii) differences in resistance correlate to altered expression levels of defence-related genes and iii) site-specific resistance can be modulated by soil management practices.

Methods

Vineyards The susceptibility of grapevine *cv* Chasselas grown in three vineyards in the area of Lake Neuchâtel was compared from 2004 to 2008. Vineyards are situated at Hauterive (HAU), Auvernier (AUV) and Concise (CON) (Table 1) and are managed and certified according to standards of organic agriculture. At all three sites, plants were sprayed with the same plant protection products that are allowed in certified organic farming in Switzerland according to similar

Table 1 Coordinates and soil properties of the three vineyards in the region of Lake Neuchâtel

		Auvernier (AUV)	Concise (CON)	Hauterive (HAU)
Coordinates		46°58′35″N, 6°52′45″E	46°51′0″, 6°43′12″E	47°0′55″N, 658′15″E
Altitude		433 m	440 m	501 m
Soil type		loam	highly loamy sand	sandy loam
Sand/silt/clay (%)		47/27/26	61/24/15	58/24/18
Mould (%)		2.6	2.3	2.1
pH		7.4	7.2	7.7
Nutrients (available/reserve)[1]	P	13/153	15/170	11/97
	K	71/354	72/212	64/262
	Mg	9/456	9/395	7/489
	Ca	154/55′164	144/56′262	153/78′982
	Cu	–/207	–/258	–/172
	Fe	–/177	–/235	–/180
Dehydrogenase activity (DHA)[2]		7.0	5.4	5.6

[1] $mg\,kg^{-1}$; [2] $mg\,g^{-1}\,h^{-1}$

spray schedules and dosages. The products are non-systemic contact fungicides (copper, sulphur) that loose efficacy after rainfall of more than 15–20 mm. Management of the vineyard at CON was changed from organic to integrated production in 2008 and was thus excluded from the analysis in 2008. The effect of long-term amendment of composts was assessed in two field-experiments, which had been established in 1996 in two organically managed vineyards (*cv.* Pinot noir) at Malans (MAL) and Walenstadt (WAL) in the eastern part of Switzerland. Soils were either not (control) or yearly amended with 50 (Walenstadt) or 40 (Malans) $m^3\,ha^{-1}$ of a compost (10–30% farm yard manure, 40–60% biodegradable waste including wood chips, 10% top soil, 10% screen overflow of compost, 1.5% rock meal) (Maschinenring Zuger Berggebiet, Edlibach, Switzerland). The identity of the cultivar Chasselas was assessed with six microsatellite markers (VVMD5, VVMD7, VVMD27, VVS2, VrZAG62 and VrZAG79), the markers of choice for grape cultivar identification (This et al. 2004). The allele lengths were entered into the Swiss Vitis Microsatellite Database (SVMD) which contains data of about 170 *Vitis* spp. accessions cultivated in Switzerland (Vouillamoz et al. 2009).

Soil samples and soil analysis Soils were randomly sampled at AUV, CON and HAU in July 2005 with a 3 cm soil corer from the 0 to 20 cm soil layer in each field plot after removing organic matter at the soil surface. Soils were sieved to 1 cm, homogenised, watered to field capacity and stored at 2°C under aerobic conditions until further analysis. Soil dehydrogenase activity (DHA) was measured according to Tabatabai (1982) in 5 g of soil that were incubated at 30°C for 24 h in the presence of an alternative electron acceptor (triphenyltetrazoliumchloride). The red coloured product (triphenylformazan) was extracted with acetone and measured in a spectrophotometer at 546 nm. Other soil parameters including pH, sand/silt/clay content, mould content, P, K, Mg, Ca, Fe and Cu content (available and reserve) were determined according to the standards of the Swiss Federal Agronomic Research Stations Agroscope (Anonymous 1996).

Leaf disc bioassays Twenty plants were sampled at each of the AUV, CON and HAU sites between May 30 and June 9 in the years 2004–2008. At sites MAL and WAL, twelve plants per site and treatment were harvested in early June 2005 and 2006. From each plant, five (AUV, CON, HAU) or three to four (MAL; WAL) leaves from a main shoot were sub-sampled, starting with the youngest leaf of approximately 2/3 of the size of a fully grown leaf (leaf age 1), and the next older leaves. To remove residues of plant protection products, leaves were thoroughly cleaned by first soaking in water containing 0.01% Tween for

3 min, washing with a shower (30 s for each side of the leaf), and drying with clean paper towels. Two leaf discs were cut per leaf (14 mm diameter), placed on 1% water agar and inoculated with 10 µl droplets of *P. viticola* sporangia suspension (5×10^4 sporangia ml^{-1}). Within one leaf age, the spread of the droplets was homogeneous and did not vary between sites (data not shown). Droplets were dried with a paper towel 24 h after inoculation to avoid necrosis. Lesion diameters were measured 7 d post inoculation. In 2007, in order to analyze gene expression, in addition to the two leaf discs for susceptibility bioassays, 9 leaf discs (10 mm diameter) were cut per leaf and placed on 1% water agar. Leaf discs were sampled 0, 8, 12, 24 and 48 h after inoculation with *P. viticola* or mock-inoculation with H_2O, separately for each site, leaf age and type of inoculation (*P. viticola*-/mock-inoculation) (i.e. 20 leaf discs from 20 plants pooled per time point and inoculation type).

Grafting experiments Scions from the three sites were harvested in winter 2006 and grafted onto rootstock Kober 5BB by a commercial grapevine nursery (Rebschulen Andreas Meier, CH-Würenlingen). Young grafted vines were either grown in the greenhouse or planted in the vineyards at sites HAU or AUV in August 2007 to compare (i) the susceptibility of scions of different origin under identical conditions and (ii) the susceptibility of scions of identical origin grown at different sites. Leaves for bioassays were harvested on June 9, 2008 and processed as described above.

Compost bioassays In order to assess the potential of composts to reduce susceptibility of grapevine to *P. viticola*, 19 composts from nine commercial compost producers were tested under controlled conditions. Composts of each of the four quality groups (digestates for agricultural use, composts for agricultural use, composts for horticultural use, compost for covered cultures and private gardening) as defined by the guidelines of the Swiss Compost Plants were used (Fuchs et al. 2001). Feedstock and processing of each of the composts was known, and over 50 parameters including chemical and physical parameters, microbial activities, plant compatibility and suppressiveness to soil-borne diseases had been determined according to Kupper and Fuchs (2007) (unpublished data). Twenty percent (v/v) of compost was thoroughly mixed with a standard substrate ('Einheitserde Typ 0', Gebr. Patzer GmbH & Co. KG, D-Sinntal-Jossa) previously amended with 3 gl^{-1} of a mineral fertilizer (Tardit 3 M, Hauert Günther Düngerwerke GmbH, D-Erlangen). Plants were fertilized every second week with an ammonium nitrate solution (NH_4NO_3, 0.024 gNl^{-1} substrate), starting at the date of planting. Seedlings were used for greenhouse assays. Small seedlings of grapevine *cv.* 'Chasselas' (Syngenta AG, Stein, Switzerland) were transplanted to the substrate and fertilized as described above. Plants were grown in the greenhouse at a temperature of 18–28°C under natural light. In winter time, photoperiod was extended with mercury lamps to 16 h. Plants were drop inoculated with a sporangia suspension of *P. viticola* when they had 6 fully expanded leaves (two 5 µl drops on each fully expanded leaf, 5×10^4 sporangia ml^{-1}). After inoculation, plants were incubated at 100% relative humidity at 20°C for 24 h, then transferred to growth chambers (60% RH, 14 h light [80 µE $m^{-2}s^{-1}$] 20°C during day, 18°C during night) for 5 days and returned to the humidity chamber the evening before scoring in order to initiate sporangia production.

The same 19 composts were tested in parallel in the bioassay *Arabidopsis thaliana* L.—*Hyaloperonospora arabidopsidis*. *A. thaliana* is known to be able to respond to appropriate organic soil amendments or beneficial soil microorganisms with altered disease susceptibility (Vallad et al. 2003; van Loon et al. 1998). *A. thaliana* seeds accession Columbia *(Col-0)* were stratified in 0.1% water agar at 4°C for 4 d and planted directly on the substrate (pots ∅ 9 cm, 275 ml), with six replicates per treatment. Seven to 10 days after planting, plants were thinned to 5 per pot. Plants were grown in a growth chamber with a 10 h day (100–120 µE $m^{-2}s^{-1}$ at 23°C) and 14 h night (18°C) cycle at 70% relative air humidity for 18 days before inoculating with *H. arabidopsidis* strain NOCO (2×10^4 conidia ml^{-1}) with a hand sprayer. Plants were then incubated at 19°C, 100% relative air humidity and 10 h light during 24 h. Subsequently, relative air humidity was reduced to 80% for 5 d, then increased again to 100% to induce sporulation before assessing disease severity and harvesting. Disease severity (percentage leaf area covered by conidiophores) was assessed for each plant separately by assessing upper and lower sides of the leaves. In both bioassays, six replicates per treatment were used. The

19 composts were assessed in two distinct experimental sets, each including an internal control (standard substrate amended with mineral fertilizer as described above). The experiment was repeated with grapevine *cv.* Chasselas cuttings (Agroscope Changins-Wädenswil, Changins, Switzerland) instead of seedlings as described above with 10 composts, five of which had shown a disease suppressive effect in the *A. thaliana—H. arabidopsidis* bioassay in previous experiments (data not shown).

Preparation of cDNAs and real-time PCR for expression analysis of VvGluc, VvLox9, VvNCED and VvSTS Total RNA was isolated from frozen leaf tissue using a modified CTAB extraction and lithium chloride precipitation method according to Iandolino et al. (2004). The quantity of total RNA was determined with a Nano-Drop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). For quantitative real-time PCR analysis, RNA was treated with Turbo-DNase I (Ambion) according to the manufacturer's instructions. For cDNA synthesis, 1 μg of RNA was reverse transcribed using oligo$(dT)_{18}$ and Superscript III reverse transcriptase (Invitrogen Life Technologies) following the instructions of the manufacturer.

Expression analysis of the genes *VvGluc*, *VvLox9, VvNCED* and *VvSTS* was done by real-time PCR, using SYBR green method on an iCycler (Bio-Rad) real-time cycler. Each PCR reaction (20 μl) contained 0.25 mM of each primer, cDNA and 1 x SYBR Green Master Mix Reagent (Bio-Rad). The thermal cycling conditions were 95°C for 3 min followed by 95°C for 30 s, 56°C for 30 s, and 72°C for 35 s for 40 cycles, followed by a melt cycle from 60°C to 95°C. The primers used were as follows: VvGluc-F (5′-ACCAC CAGCATCACAAGTGGTA-3′) and VvGluc-R (5′-TGTTAAGTCGATTGCGGTGGAG-3′) for *VvGluc*, VvLox9-F (5′-TCAGTTCTGGTGGAAGGAAGT-3′) and VvLox9-R (5′-AGGCATGAATCTGCGGCT TATC-3′) for *VvLox9,* VvNCED-F (5′-TGATG TGGTCCAGAAGCCATAC-3′) for *VvNCED* and VvStSy-F (5′-CTCGAACCATCCGTCAGAAGAG-3′) and VvStSy-R (5′-CCTACGATTACAGCTGCA GACC-3′) for *VvSTS*. With all cDNAs used, the above primer sets gave single PCR products, which were verified by determining the melt curves for the products at the end of each run and by analysis of the products using gel electrophoresis. The efficiency of the primers was tested in preliminary experiments with serial dilutions of cDNA samples and maintained an E value of between 0.97 and 0.98. The expression of the four genes was normalised relative to Elongation Factor 1-α (*VvEF1-α*). All samples were measured in triplicate, every run included the *VvEF1-α* control for each sample. The Gene-X software (Bio-Rad) was used to calculate the mean normalised expression of the genes.

Statistical analysis Susceptibility of grapevine seedlings and *A. thaliana* plants grown in different composts or in the control were compared by an ANOVA (α=0.05) followed by a Tukey-B test at α= 0.05 in case of significant results. Variation in the gene expression pattern of the four defense-related genes was evaluated separately for each timepoint after inoculation by a constrained linear ordination using Redundancy Analysis (RDA) with the environmental factors site, leaf age, and type of inoculation (mock-inoculation/inoculation *with P. viticola*), and results were visualized in an ordination diagram (Software CANOCO 4.5).

Results

Effect of site on the susceptibility of grapevine to *P. viticola*

The susceptibility of grapevine *cv.* Chasselas to *P. viticola* grown at three sites in the region of Lake Neuchâtel was studied in the years 2004 to 2008. The three vineyards are located a maximum of 26 km apart in the same geographic region (south-east facing slopes at the north-west shore of Lake Neuchâtel) (Table 1). Soils at the three sites were similar in their contents of available and reserve P, K, Mg, Ca and Fe, but differed in their sand-silt-clay contents, the percentage of stones and in soil depth (Table 1). Grapevine grown at the three sites differed significantly in their susceptibility to *P. viticola* in all 5 years of examination (Fig. 1). In 4 out of 5 years, plants from the HAU site were much less susceptible than plants from site AUV. In another year (2005), differences between HAU and AUV were less pronounced but still significant for one leaf age (Fig. 1). Susceptibility of plants from the CON site varied from year to year, being either as resistant as plants from the HAU site (2004), nearly as (2007), or

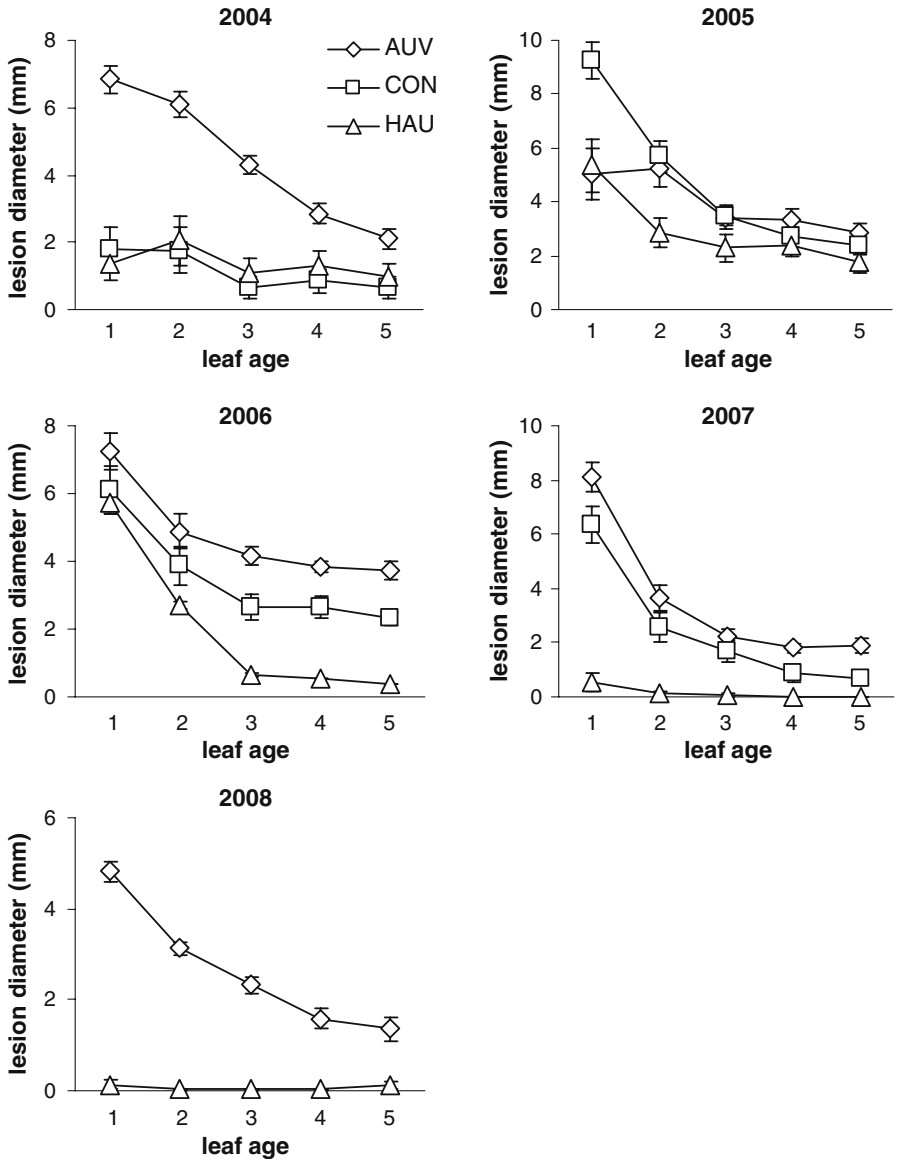

Fig. 1 Downy mildew susceptibility of grapevine leaves from plants (*cv.* Chasselas) grown in three commercial organic vineyards in the area of Lake Neuchâtel (AUV, CON and HAU) in five successive years. At each location, leaves of twenty plants were sampled, starting with a leaf of approximately 2/3 the size of a fully grown leaf (leaf age 1), and the next four older leaves. After washing thoroughly, two leaf discs were cut from each leaf, and inoculated with *P. viticola*. Lesion diameters were measured 7 d post inoculation. The figures show means±SE

even more (2005) susceptible than plants from the AUV site (2007) or intermediate (2006). Growth status of grapevine was comparable in all three sites (data not shown). Microsatellite analysis revealed that plants from all three sites have the same genetic background. Furthermore, grafted vines produced from scions of plants of each of the three sites did not differ in their susceptibility to *P. viticola* if grown either in the vineyard at the HAU site or in a standard substrate in the greenhouse (Fig. 2a). In contrast, grafted plants from the three sites grown at the AUV site tended to be more susceptible to *P. viticola* than plants grown at the HAU site in the first growing season after planting (Fig. 2b).

Effect of site on the expression pattern of four selected defence-related genes

In 2007, the expression patterns of four selected defence-related genes (β-1,3-Glucanase (*Glu*), stilbene synthase (*STS*), lipoxygenase 9 (*Lox9*) and 9-cis-epoxycarotenoid dioxygenase (*NCED*)) in leaves from the three sites and in five leaf ages were studied. Redundancy analysis on the constitutive expression

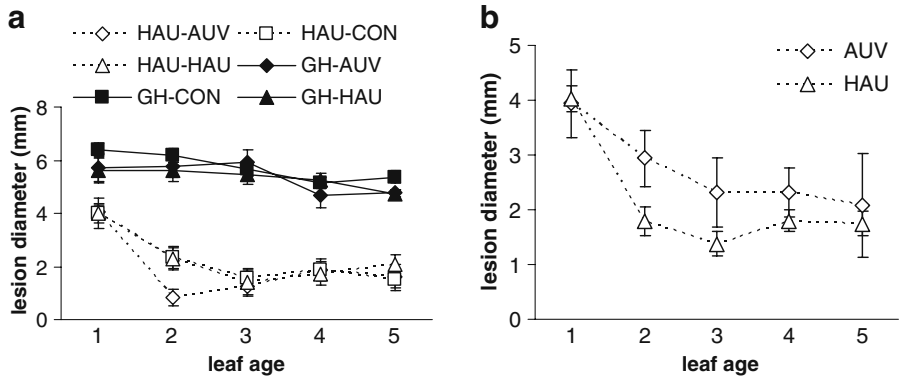

Fig. 2 Downy mildew susceptibility of young grafted vines (*cv.* Chasselas) produced from scions of three vineyards (AUV, CON and HAU) (grafted onto rootstock 5BB). Figures show (**a**) susceptibility of grafted vines produced from scions of the three origins grown for 9 months either in the greenhouse (GH) (filled symbols, solid lines) or in the field at site HAU (empty symbols, dashed lines) or (**b**) susceptibility of grafted vines produced from scions from the three vineyards and planted at sites AUV or HAU, in their first season after planting (data pooled for each site). From each plant, two leaf discs per leaf were cut from 5 leaf ages, starting with a leaf of approximately 2/3 the size of a fully grown leaf (leaf age 1), and the next four older leaves. Leaf discs were inoculated with *P. viticola* and lesion diameters measured 7 d post inoculation. The figure shows means±SE, n=25 (HAU), 10 (GH-AUV, GH-CON, GH-HAU), 9 (HAU-AUV, HAU-CON), 7 (HAU-HAU) or 4 (AUV). Legends indicate (**a**) site of growth—origin of scions or (**b**) site of growth

pattern revealed that 41% of the total variance was explained by the factor site (p=0.002), and 24% by the factor leaf age (p=0.014) (in total 65% explained variance) (Tab. 2). Visualisation of the results showed a clear separation of the HAU site from the other two sites (Fig. 3). High expression levels of *Glu* and *Lox9* were associated with samples from the HAU site and positively correlated with resistance to *P. viticola*. The expression level of *NCED* was mainly correlated with leaf age, with increased levels in older leaves. *STS* had only a minor impact on the ordination (Fig. 3). In addition to constitutive expression levels, the four genes were monitored for 48 h after cutting leaf discs and inoculation. Redundancy analysis revealed that (i) total variance explained by the factors site, leaf age and type of inoculation decreased from 65% to 25% over time (ii) percentage variance explained by the factor site decreased over time from 41% to 12%, but remained significant, and (iii) after 12 h and later on, inoculated and non-inoculated leaf discs did not differ in their expression patterns (Table 2).

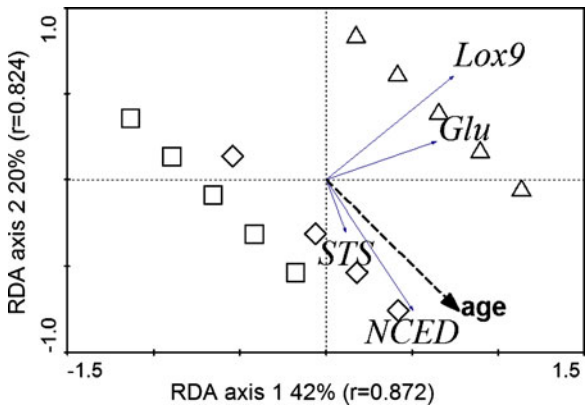

Fig. 3 Distribution of gene expression levels of grapevine before inoculation with *P. viticola*, in the ordination biplot of a redundancy analysis (RDA). Samples were labelled by site (triangles AUV, diamonds CON, circles HAU). The vectors represent the individual genes (solid vectors) and the environmental factor leaf age (dashed vector). Expression levels are relative to expression level of *VvEF1-α*, and the data were log-transformed

Modulation of site-specific susceptibility of grapevine to *P. viticola* by the application of composts

The effect of the long-term application of compost on resistance of grapevine *cv.* Pinot noir against *P. viticola* was examined in two field experiments, which had been established in 1996. After 10 years of continuous compost amendment, no significant differences between grapevine plants grown in compost-treated or control plots were detected in two consecutive years of sampling (Fig. 4b). Yet,

Table 2 Variance in the expression level of four defence-related genes before inoculation (constitutive expression level, t=0) and 8, 12, 24 and 48 h post (mock-) inoculation (p.i.) as explained by the factors site, leaf age, and inoculation type (mock- or pathogen-inoculation) determined by redundancy analysis

Factors	Explained variance				
	Constitutive expression (t=0)	t=8 h p.i.	t=12 h p.i.	t=24 h p.i.	t=48 h p.i.
All factors	64.6%	53%	44%	25%	39%
Sites	41% *	31% *	23% *	12% *	14% *
Leaf age	23.6% *	11% *	18% *	7% *	20% *
Inoculation	–	10% *	1%	3%	3%

* significant for all canonical axes, Monte Carlo Permutation Test, $p<0.05$

grapevines grown at the WAL site were less susceptible to *P. viticola* than plants grown at the MAL site in both years of examination (Fig. 4). To further study the effect of composts on the resistance of grapevine to *P. viticola*, 19 different composts were evaluated in the greenhouse on grapevine seedlings *cv.* Chasselas (Fig. 5a). As a control, the same 19 composts were evaluated in the system *A. thaliana-H. arabidopsidis*. None of the 19 composts had a significant effect on the resistance of grapevine seedlings. Interestingly, 8 of the 19 composts significantly reduced disease severity in the control system *A. thaliana- H. arabidopsidis* (Fig. 5b), and these results were repeatable (data not shown).Testing ten selected composts, eight of which had shown significant effects in the *A.thaliana-H.arabidopsidis* control system in previous experiments, on grapevine cuttings gave similar results as on grapevine seedlings (data not shown).

Discussion

Our research points to a substantial influence of site-specific properties on field resistance of *cv.* Chasselas and *cv.* Pinot noir. In the case of Chasselas, differences in resistance between the different investigated sites were substantial and relevant for commercial wine production, with leaves grown at the HAU site hardly developing disease symptoms upon inoculation with *P. viticola* in 3 of the 5 years examined. Such large site-specific differences in suppressiveness have been reported before, mainly for suppressiveness to soil-borne diseases (e.g. reviewed by Weller et al. (2002)). In addition, some studies showed that site-specific factors can have a similarly high impact on the resistance of plants to air-borne diseases. For instance, *A. thaliana* plants grown on different soil types differed in their susceptibility to downy mildew caused by *H. arabidopsidis* by a factor of six (i.e.

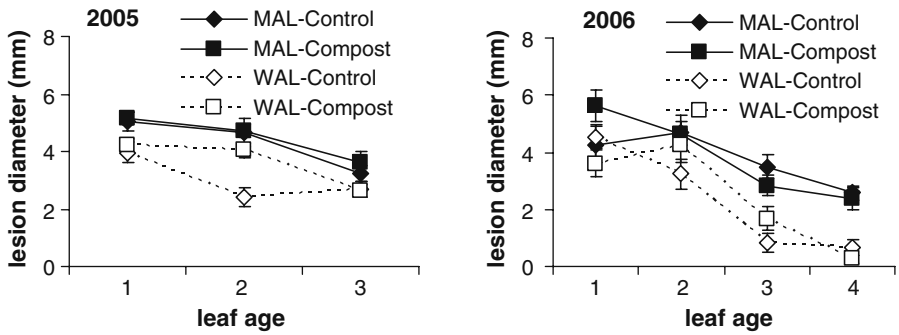

Fig. 4 Downy mildew susceptibility of grapevine *cv.* Pinot noir grown in two vineyards at Malans (MAL) or Walenstadt (WAL) amended or not-amended (control) with a compost after nine (2005) (**a**) or 10 years (2006) (**b**) of compost application. At each location, and in each treatment, leaves of twelve plants were sampled, starting with a leaf of approximately 2/3 the size of a fully grown leaf (leaf age 1), and the next two or three older leaves respectively. After washing thoroughly, ten leaf discs were cut from each leaf and inoculated with *P. viticola*. Lesion diameters were measured 7 d post inoculation. The figures show means±SE

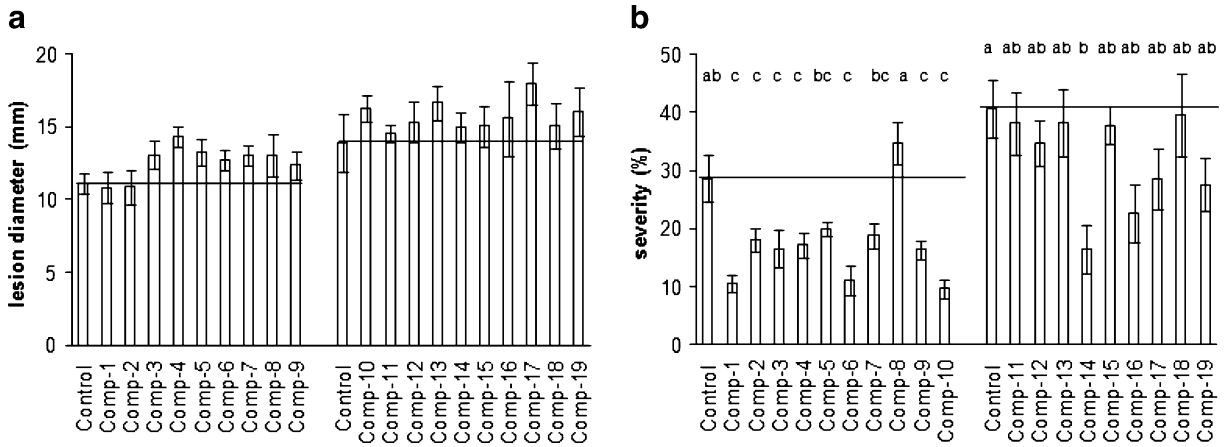

Fig. 5 Susceptibility of a) seedlings of grapevine *cv.* Chasselas to *P. viticola* and b) *A. thaliana* to *H. arabidopsidis* grown in a substrate amended with one out of 19 different composts (comp-1 to -19) or in the standard substrate only amended with a mineral fertilizer (control). The 19 composts were tested in two distinct experimental sets, each with the same internal control. *A. thaliana* plants were sprayed with *H. arabidopsidis* at the size of 5 true leaves. The area covered with conidiophores was assessed 7 d post inoculation. Each grapevine leaf was inoculated with 2 drops of *P. viticola,* and lesion diameters were measured 7 d post inoculation. The figures show means± SE, n=6. The treatments were compared by an ANOVA (α= 0.05) followed by a Tukey-B test (α=0.05) in case of significant differences

plants grown on the most suppressive soil were six times less infected than plants grown on the least suppressive soil), whereas tomatoes grown on the same soils only differed by a factor of 0.2 (Tamm et al. 2010). To our knowledge, no such relevant differences between sites have been reported before for resistance of woody perennial plants such as grapevine to air-borne diseases. Although the site, i.e. the terroir, has been shown before to have a great impact on grape berry quality including the contents of many plant metabolites and on the resulting vine (Jackson and Lombard 1993; Morlat and Bodin 2006; Seguin 1986), the relative contribution of site-specific factors such as various soil parameters, climate, plant material, and anthropogenic impacts to the differentiation between sites is difficult to evaluate, mainly because of interactions between the different parameters (Seguin 1986). Nevertheless, soil has been identified as a key determinant by many studies, affecting various parameters relevant for grape berry and vine quality, such as mineral nutrient availability, water holding capacity and rooting depth (i.e. water uptake conditions), or the temperature in the root zone (Downey et al. 2006; Morlat and Bodin 2006; Seguin 1986).

Here we show that differences in susceptibility to *P. viticola* were correlated to altered constitutive gene expression patterns, even though only four defence-related genes were investigated. Studying the expression pattern of more defence-related genes might even lead to a further differentiation, e.g. between sites AUV and CON. The four genes were selected as representatives of different signalling pathways known to be involved in constitutive or induced resistance of different plants. Plants grown at the HAU site showed increased constitutive expression levels of *Lox9* and *Glu*. *Lox9* catalyses the rate-limiting step in jasmonic acid (JA) biosynthesis. JA has been shown to be involved in resistance of *P. viticola*-resistant varieties such as Solaris, but also in BABA-induced resistance (Hamiduzzaman et al. 2005). *Glu* is a marker gene of the salicylic acid (SA) pathway. As *Lox9*, its expression can be induced or potentiated by BABA or laminarin (Aziz et al. 2003; Hamiduzzaman et al. 2005). Yet, activation of the SA-pathway has been reported not to be sufficient in enhancing resistance of grapevine to *P. viticola*. Increased leaf age was not only correlated to increased levels of resistance to *P. viticola*, but also to increased expression levels of *NCED*, which is involved in the synthesis of abscisic acid (ABA). ABA was not effective in potentiating resistance of grapevine *cv.* Chasselas in leaf disc experiments (Hamiduzzaman et al. 2005), but can induce resistance against necrotrophic pathogens in *A. thaliana* (Ton and Mauch-Mani 2004). In contrast to differences in the constitutive expression pattern,

there was no evidence that the four defence-related genes are activated faster and/or stronger upon contact with *P. viticola* in plants from the HAU site than in plants from any of the two other sites, as could be expected in classical soil microorganism-induced systemic resistance. Yet, 12 h after cutting the leaf discs and mock inoculation, the expression pattern of *P. viticola*—inoculated leaf discs did not differentiate from mock-inoculated leaf discs and the percentage of unexplained variance steadily increased. These results suggest that the cutting of leaf discs imposes substantial stress to the plants, and that the plant's reactions to this stress might overlay other reactions such as a putative priming effect. Thus, to monitor the reaction of the plants over a prolonged time-period, using whole leaves or whole plants instead of leaf-discs might give more detailed results. However, to compare plants grown in the field, using whole plants is not a feasible approach.

For an application in commercial agriculture, it is highly relevant whether site-specific suppressiveness of soils can be modulated by management techniques such as the application of organic amendments. Several studies have demonstrated that suppressiveness of natural soils to soil-borne, but also to air-borne diseases can be affected by the application of organic soil amendments such as manure and compost (Fuchs et al. 2004; Litterick et al. 2004; Tamm et al. 2010; Vallad and Goodman 2004). However, the effect of soil-amendments on resistance of various plant species to soil- and air-borne diseases was much smaller than site-specific effects (Tamm et al. 2010). In the present study, we found no evidence that the resistance of grapevine to *P. viticola* can be influenced by the application of composts. In two field experiments, no difference in the resistance of grapevine plants to *P. viticola* grown in control- or compost-treated plots were found, neither in the field (data not shown), nor when leaves were tested under controlled conditions in the lab, even though compost had been applied before annually for 12 years. Composts are known to differ in their quality and their ability to suppress soil-borne diseases depending on their composition, the production process, and maturity (Fuchs et al. 2004). Therefore, we have also evaluated the potential of 19 composts with different chemical, physical and microbial properties to reduce the susceptibility of grapevine seedlings and cuttings under controlled conditions. None of them had any effect in the system grapevine-*P. viticola*, even though eight of them significantly reduced disease of *A. thaliana* with *H. arabidopsidis*, our positive control known to be inducible via the root system. These results indicate that, in contrast to other plant-pathogen systems, it is unlikely to positively modulate site-specific resistance of grapevine plants to *P. viticola* by the application of composts.

In conclusion, we have shown that site has a major impact on the resistance of grapevine to *P. viticola*. Differences in resistance were correlated to altered expression patterns of defence-related genes, indicating that induced resistance might be the underlying mechanism for the observed differences in disease resistance. However, more research is needed to identify the site-specific factors involved in inducing resistance. In this study, site-specific suppressiveness to *P. viticola* could not be modulated by the application of composts, making an exploitation of the phenomenon in commercial agriculture unlikely.

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