Vitis **32**, 69—76 (1993)

Relations between replant disease, growth parameters and mineral nutrition status of grapevines (*Vitis* sp.)

by

CHRISTINA WASCHKIES[1]), A. SCHROPP[1]) and H. MARSCHNER[2])

[1]) Landes-Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau, Neustadt/W., Deutschland

[2]) Institut für Pflanzenernährung, Universität Hohenheim, Stuttgart-Hohenheim, Deutschland

Summary: Grapevine cultivars Riesling (*Vitis vinifera* L.), 5 C (*V. berlandieri* PLANCH. x *V. riparia* MICHX.), Riesling grafted on rootstock cultivar 5 C (Riesling/5 C) and maize (*Zea mays* L.) were grown in pots on a soil from a grapevine nursery affected with replant disease and on a soil that had not been planted with grapevines before. On replant soil the grapevines, in particular 5 C and Riesling/5 C, were severely inhibited in shoot and root growth, while ungrafted Riesling was less affected. In contrast, maize produced similar dry weights on both soils.

There was no causal relationship between the incidence of replant disease in grapevines and their nutritional status, regarding macro- and micronutrients. However, compared to non-replant soil, on replant soil the manganese concentrations in shoots of all grapevine types were significantly lower, indicating an alteration of microbial activity and/or microbial composition in the rhizosphere of grapevines.

Grapevine replant disease could not be induced in a soil by incubating the tenfold amount of grapevine roots, that seasonally remain in the nursery soil. Thus, the growth inhibition of grapevines cannot be attributed to substances released from dead grapevine roots of the degradation products. Obviously, factors associated with living roots (rhizosphere) are an essential prerequisite for the development of replant disease. The elimination of replant disease by steam sterilization of replant soil supports the assumption, that rhizosphere microorganisms are involved with grapevine replant disease.

Key words: Vitis, nursery replant problems, mineral nutrition, manganese, root residues, autotoxicity, soil sterilization.

Introduction

As a rule the production of vigorously growing grapevines in nurseries is possible on the same field for only three or four seasons. Even after intercropping other plant species for several years, the subsequently replanted grapevines may be severely impaired in growth, showing stunting and damaged root systems. A delayed initial development of grapevines may also occur when old vineyard sites are re-established. Replant problems are independent of the soil type, and at present only nematodes could be excluded as a causal factor (SCHUMANN and RÜDEL 1982). Toxic compounds have been implicated in replant problems of grapevines (MOSER 1956; BRINKER and CREASY 1988) and other woody species (PATRICK 1955; BÖRNER 1963; BURGER and SMALL 1983). The incidence of replant problems seems to have accelerated since polyethylene mulches are used in the planting rows which might decrease the leaching of toxic compounds to the subsoil. On the other hand this practice may affect also soil temperature, soil moisture and mineral nutrition of the grapevine cuttings. In the present study relationships between growth inhibition and nutrient status of grapevines on replant soil and increasing quantity of root residues in nursery soils are investigated.

Correspondence to: Dr. CHRISTINA WASCHKIES, Landes-Lehr- und Forschungsanstalt für Landwirtschaft, Weinbau und Gartenbau, Breitenweg 71, D-67435 Neustadt/W., Germany.

Materials and methods

Soils: *Replant soil* (RS) was collected in December 1987 from a nursery field that had been used for cultivation of grapevines for 4 years and where severe growth inhibition of grapevine cuttings had occurred in the previous growing season. *Non-replant soil (NRS)* was collected from a nearby grassland that had not been planted with grapevines before. The soils were sieved (< 5 mm), stored at 2.5 °C and fertilized with N, P, K and Mg to obtain a similar nutrient status in both soils (see Tab. 1 for further data). After fertilization the soils were sieved again (< 5 mm) and filled in the pots. Steam sterilized soils were produced by autoclaving RS and NRS at 121 °C and stored for 8—10 d before use.

A third soil — *Incubated soil* (IS, Tab. 1) — was collected from a field with long-term cereal cropping that had not been planted with grapevines before. Chopped roots (R) of grapevine 5 C (1—2 cm length) were thoroughly mixed with the soil at a rate of 4 g of roots per kg soil. This rate corresponded to about the tenfold amount of grapevine roots remaining each year in the soil of the nurseries. This soil (IS + R) and the soil without roots (IS) were brought to about 70 % field capacity and incubated for 4, 20 or 31 months at 25—27 °C in the greenhouse.

Table 1 : Chemical and physical properties of the soils used in the experiments

Soils	Org.matter	pH	P	K	Mg	B	Physical analysis [%]		
		(KCl)	-(CAL)-		($CaCl_2$)	(H_2O)	clay	silt	sand
	[%]		[mg $100g^{-1}$ soil]				[%]	[%]	[%]
Replant soil (RS)*	2.0	7.6	23.1	23.2	9.5	1.3	18.4	56.9	24.7
Non-replant soil (NRS)*	2.4	7.4	14.8	29.8	12.0	1.6	19.5	43.2	37.3
Incubated soil (IS)	2.4	7.4	18.5	13.1	8.0	1.0	7.1	65.4	27.5

*Soils received 5 mg N $100g^{-1}$ soil as $Ca(NO_3)_2$.
$Ca(H_2PO_4)_2$, K_2SO_4 and $MgSO_4$ were supplied to obtain final concentrations of 23 mg P $100g^{-1}$, 30 mg K $100g^{-1}$ and 23 mg Mg $100g^{-1}$ soil.

Cultivation of plants, plant harvest, and analysis: Two-bud cuttings of cultivar 5 C [6 Gm] (*V. berlandieri* × *V. riparia*), three-bud cuttings of cultivar Riesling [N 90] (*V. vinifera* L.) both of one-year-old wood, and grafted Riesling on rootstock 5 C (Riesling/5C) were rooted in moist peat. Two-bud cuttings of green shoots of 5 C (green cuttings) were rooted in moist perlite for 4 weeks. All experiments were carried out in a greenhouse at 25 ± 3 °C/22 ± 3 °C at day (16 h)/night (8 h) and supplemented with sodium vapour lamps (Osram HQI) for 16 h.

Experiment 1: At an average shoot length of 10 cm the plants were transferred to plastic boxes (50 × 37 × 9 cm) filled with 16.7 kg of air-dry soil (RS or NRS), and brought to bulk density of 1.0 g cm^{-3}. Constant supply with tap water was maintained by glass fibre wicks. Two cuttings of the same genotype (Riesling/5 C, 5 C and Riesling) were planted in each box (two pots of each treatment) and harvested after 54 d. To control powdery mildew Bayleton [Triadimefon] 0.075 % was sprayed once. At the harvest the root systems were washed free from soil. Total shoot length, distance between apex and first full-grown leaf (= 'youngest shoot'), the average internode-length, shoot and root dry weights (after 80 °C, 48 h) were determined. Dried samples were ground, dry-ashed at 500 °C and digested with HCl (10 %). Phosphorous

was determined colorimetrically (GERICKE and KURMIES 1952), K and Ca by flame photometry and Mg, Fe, Zn and Mn by atomic absorption spectrometry. Nitrogen was determined separately with the Kjeldahl method.

To test the plant species specifity of replant disease, the RS and NRS were used in a pot experiment with maize (*Zea mays* L. cv. Gelber Badischer Landmais, one plant per pot in 850 g of soil, 6 replications per soil type). Shoot dry weight was recorded after 30 d of growth.

Experiment 2: Green 5 C cuttings and 5 C cuttings from one-year-old wood were planted in pots containing RS or NRS. For green cuttings pots with 600 g of soil, for the woody cuttings pots with 1.8 kg of soil were used (5 replications per treatment). Manganese concentrations in the leaf blades were determined after 10 d of growth (green cuttings) and after 19, 42 or 62 d (woody cuttings).

Experiment 3: Green cuttings of 5 C were grown in pots with 600 g IS+R or IS (5 replications per treatment for the pot trial after 4 months of incubation, 10 for the trials after 20 and 31 months). Shoot length was measured weekly during the experiment.

Experiment 4: The same as experiment 3 but with sterilized RS and NRS (5 replications). Dry weights were recorded after 95 d.

Soil analysis: Water extractable Mn in soils was determined by shaking 10 g of air dried soil in 100 ml demineralized H_2O for 1 h; for other methods see Tab. 1.

Statistical analysis: Analysis of variance of the data was carried out using Scheffé's test. The least significant difference were calculated at $P = 0.05$.

Results

Compared to non-replant soil (NRS), grapevine growth was severely impaired on replant soil (RS) (Tab. 2). In contrast, maize produced similar dry weights on both

Table 2: Effect of replant soil (RS) and non-replant soil (NRS) on growth parameters of grapevines and of maize[a]

Soil	Plant genotype	Dry weight shoot [g plant^{-1}]	Dry weight roots [g plant^{-1}]	Internode length* [cm]	Youngest shoot** [cm]
RS	5C	2.7 a	0.46 a	2.6 a	6.0 a
NRS	5C	12.2 b	1.80 b	5.7 b	43.2 b
RS	Riesling	6.0 a	1.08 a	3.6 a	25.5 a
NRS	Riesling	9.1 a	1.90 a	5.1 b	32.6 a
RS	Riesling/5C[b]	1.4 a	0.20 a	1.8 a	5.0 a
NRS	Riesling/5C	9.7 b	1.87 b	4.8 b	39.0 b
RS	Maize	2.9 a	-	-	-
NRS	Maize	3.5 a	-	-	-

[a] values within a column (same plant) followed by the same letter are not significantly different (P=0.05)
[b] grafted Riesling with rootstock 5C
* average length per internode of the main shoot
** distance between first full-grown leaf to terminal bud

soils. The extent of growth inhibition on RS differed markedly between Riesling, Riesling/5 C and 5 C. The dry weights of shoot and roots of Riesling were lower on RS but not significantly different from NRS.

Figs. 1 and 2 illustrate shoot and root growth of Riesling/5 C after 54 d of growth on both soils. On roots (5 C) isolated from RS numerous lesions and sections with necrotic cortex (Fig. 3) occurred.

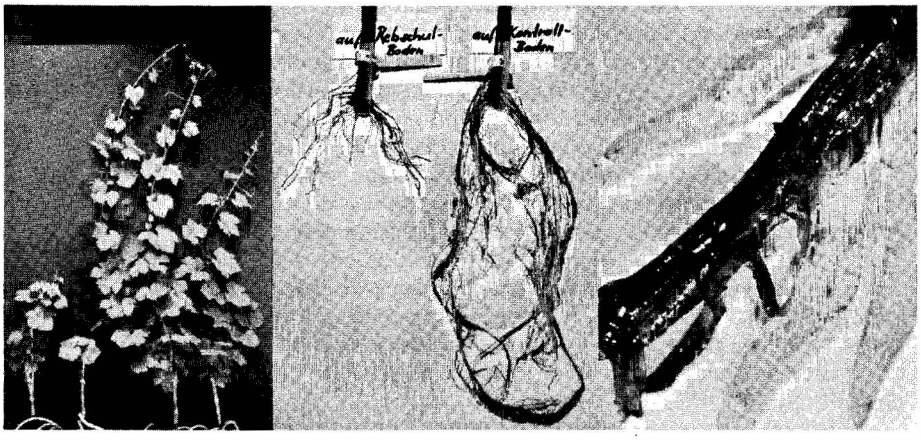


Fig. 1 (left): Shoot growth of Riesling grafted on rootstock 5 C on replant soil (left) and non-replant soil (right).

Fig. 2 (middle): Root systems of Riesling grafted on rootstock 5 C in replant soil (left) and in non-replant soil (right).

Fig. 3 (right): Details of the root system of Riesling grafted on rootstock 5 C grown in replant soil.

Concentrations of macro- and micronutrients in shoots of grapevines are shown in Tab. 3. Of the macronutrients the concentrations of K and P were somewhat lower in RS-grown grapevines. For P part of the differences are likely due to the supply of (water soluble) P fertilizer to NRS in order to adjust the levels of CAL-extractable soil P in both soils (Tab. 1). At any rate, the levels of all macro- and micronutrients (including Mn) in the shoot dry matter (Tab. 3) were in the sufficiency range on both RS and NRS, despite of the large differences in dry matter production (Tab. 2). Compared to NRS the Mn concentrations in shoots from RS were significantly lower (Tab. 3). This, along with severely depressed shoot dry weights reveals a strongly impaired Mn uptake in grapevines on RS. In experiment 3 on RS the Mn concentrations in leaves tended to decline already after 10 d compared to NRS and were significantly lower from 19 d onwards (Fig. 4).

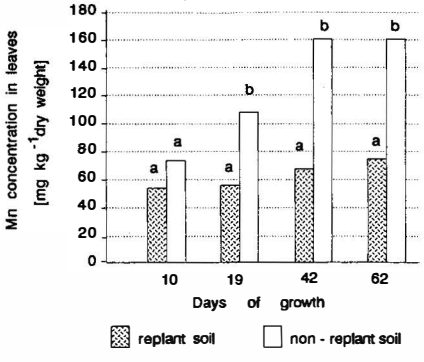
The effect of soil incubation with grapevine roots (IS+R) on shoot length of grapevine 5 C is shown in Fig. 5. Irrespectively of the length of incubation, replant disease symptoms (like stunting of shoots) could not be induced. The shoot length of 5 C was not significantly affected by the addition of grapevine roots (5 C) compared to soil incubated without roots (IS) at any time after soil incubation.

Steam sterilization of RS significantly increased shoot dry weight of 5 C (Fig. 6). In contrast, steam sterilization of NRS tended to decrease dry weight of 5 C compared to the untreated soil. Steam sterilization increased drastically the water extractable Mn 4 d after sterilization (Tab. 4). Even after 5 weeks the concentrations of water extractable Mn were still higher compared to the untreated soils. Since the soils were used in the growth experiment already 8—10 d after sterilization, excessive Mn uptake in grapevines can be assumed on the sterilized soils.

Table 3: Concentration of macro- and micronutrients in shoots of grapevines on replant soil (RS) and non-replant soil (NRS) after 54 days of growth

Soil	Plant	N	P	K	Ca	Mg	Zn	Fe	Mn
		[%]	[mg g^{-1}]				[mg kg^{-1}]		
RS	Riesling/5C*	2.07	2.68	17.7	22.0	3.43	37	139	69
NRS	Riesling/5C	1.81	3.41	22.3	19.1	2.94	24	124	106
RS	5C	1.97	1.90	13.9	21.3	3.58	30	101	55
NRS	5C	1.73	3.77	19.7	18.0	2.56	19	103	89
RS	Riesling	1.85	2.21	25.1	17.4	3.03	32	100	60
NRS	Riesling	1.66	3.20	24.3	17.7	3.40	24	107	117
	LSD (P=0.05)	0.76	1.21	6.14	4.61	0.89	23	76	38

* Grafted Riesling with rootstock 5C



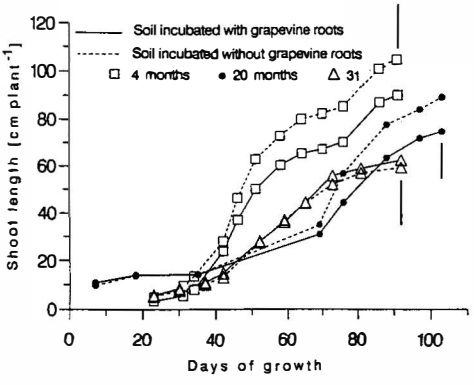


Fig. 4 (left): Concentrations of manganese in leaves of grapevine (5 C) grown in replant soil and non-replant soil. Columns with the same letters are not significantly different (P = 0.05).

Fig. 5 (right): Effect of incubating a soil with grapevine roots (5 C) on shoot length of grapevine (5 C). Vertical bars represent LSD (P = 0.05).

Discussion

Growth inhibition on grapevine nursery soil is a phenomenon specific to grapevines (Tab. 2). But also within grapevines different responses on replant soil (RS) occurred between *Vinifera* type (Riesling) and *Berlandieri* × *Riparia* type (5 C). Our results are in agreement with the observations of MOSER (1956, 1963), that grafted vines showed stunted growth in the nursery while ungrafted vines grew vigorously.

Table 4: Effect of steam sterilization on concentration of water soluble manganese in soils

	Mn concentration [mg kg^{-1} soil]	
	replant soil	non-replant soil
untreated soil	0.3	0.4
4 days after sterilization	6.8	5.0
5 weeks after sterilization	1.4	1.2

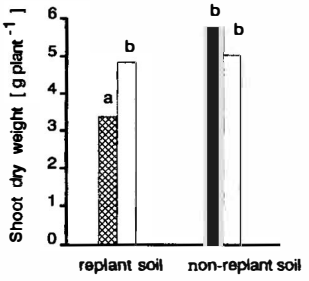


Fig. 6: Influence of steam sterilization (white columns) of replant soil and non-replant soil on shoot dry weight of grapevine (5 C); hatched columns: untreated soil. Columns with the same letters are not significantly different (P = 0.05).

Since in the production of vine plants only American rootstocks were used, mostly of *Berlandieri* x *Riparia* type, the soils of nursery fields develop replant disease affecting the rootstocks. The different growth responses of Riesling and Riesling/5 C on RS indicate that the roots play the crucial role in growth inhibition. The distinct sensitivity of Riesling and 5 C on RS is also an indication for specifity of grapevine replant disease within the genus *Vitis*.

Growth differences between RS and non-replant soil (NRS) could not be explained in terms of mineral nutrition (Tab. 3). However, the decrease in Mn concentrations in shoots on RS indicated changes in the rhizosphere soil. The availability of Mn is a function of soil chemical, plant and microbial factors (MARSCHNER 1988). RS and NRS were very similar in their chemical and physical properties (Tab. 1). It is not likely that the lower Mn concentrations in shoots of grapevines on RS were caused by changes in root metabolism such as decline of root reducing capacity or increase of rhizosphere pH. Such changes should have led to a lower acquisition of both, Mn and Fe (MARSCHNER *et al.* 1986). However, concentrations of Fe in grapevines were not affected by replant disease. Thus, the differences in Mn concentrations are most likely to be attributed to an alteration of microbial activity and/or microbial composition in the rhizosphere of grapevines on RS.

Decrease of P and K concentration in potato plants after frequent potato cropping has been found by SCHIPPERS *et al.* (1987), although the availability of P and K in the soils was not altered. In tendency, also in grapevines the concentrations of P and K in the shoots decreased in the order Riesling < Riesling/5 C < 5C (Tab. 3), i.e. in the same order as the severity of replant disease (Tab. 2). SCHIPPERS *et al.* (1987) attributed the decrease in uptake of K to an impaired root metabolism. In view of the prominent role of the root surface area for uptake of K (CLAASSEN and JUNGK 1984) and P (JUNGK and CLAASSEN 1989) it is more likely that the lower K and P concentrations in shoots are at least in part the result of root growth inhibition on RS.

Phytotoxins and allelopathic plant substances released from plant (root) residues or by exudation are suggested to be primary causal agents in replant disease of fruit

species such as peach (PATRICK 1955; HIRANO 1968), apple (BÖRNER 1959) and citrus (BURGER and SMALL 1983). BRINKER and CREASY (1988) concluded that grapevine roots are the source of at least one compound that is toxic to plants and accumulates in soil in which grapevines are grown. Reports concerning effects of root residues on plant growth are contradicting. While the results of SCHANDER (1955) and HIRANO (1968) suggest that replant diseases of peach and apple are caused by autotoxicity of root residues, this could not be confirmed by other authors (SAVORY 1969; HEIN 1972). DEAL *et al.* (1972) found that addition of grapevine roots to the soil even increased fresh weights of grapevines. The quantity of plant material added to the soils may contribute to the contradicting results on allelopathic effects of root residues or their role in replant disease. In grapevine nurseries, at the end of the season the plants are removed with root systems of approximately 50 cm length. Thus, only a minor part of the roots, estimated to be 20 % of the total root weight, remains in the soil. Addition of even an approximately tenfold amount of roots to a healthy soil was without significant effect on shoot growth (Fig. 5). This suggests that substances released from dead grapevine roots or the degradation products of roots, cannot be regarded as a major factor causing replant disease of grapevines. It is concluded that living grapevine roots are an essential prerequisite for the development of replant disease in grapevine nurseries.

The growth of grapevines on RS was significantly improved by soil sterilization (Fig. 6). The growth promoting effect of steam sterilization or chemical desinfection of soil affected with replant disease has been described before (HEIN 1972; OTTO 1972; KÜMMELER 1981). Although an influence of steaming on thermolabile allelochemicals in soil cannot be excluded, the elimination of deleterious microorganisms is the more probable explanation for plant growth promotion of grapevines. The lower dry weight of grapevines growing in autoclaved NRS (Fig. 6) was probably due to excessive uptake of Mn, as Mn toxicity may occur in plants on freshly steamed soils (BOYD 1971). Thus, the beneficial effect of sterilization of RS was probably underestimated because of the simultaneously negative effects of Mn toxicity.

In conclusion, biotic factors in the rhizosphere seem to be causally involved in replant disease in grapevine nurseries (WASCHKIES *et al.*, in preparation).

Acknowledgements

We thank the „Forschungsring Deutscher Weinbau (FDW) bei der Deutschen Landwirtschaftsgesellschaft" and the „Wiederaufbaukasse Rheinland-Pfalz (WAK)" for financial support of the studies.

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Received November 2, 1992