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FIRST EXPERIENCES WITH GRAPE ROOTSTOCKS × PHYLLOXERA INTERACTIONS

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Abstract

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Grape phylloxera, *Dactulosphaira vitifoliae* Fitch., is one of most destructive insect pest of cultivated grapes (*Vitis vinifera* L.) worldwide. Phylloxera was the cause of incomparable structural changes in European viticulture. It also led to the usage of new biotechnology – rootstocks for grape varieties. The direct damage is related to phylloxera feeding of roots and leaves. Rootstocks bred solely with American *Vitis* spp. parentage allow little or none of phylloxera related root damage that is seen on *Vitis vinifera*. Rootstocks with partial *Vitis vinifera* parentage may also confer a high level of grape phylloxera resistance, but this resistance is not durable. Rootstock was evaluated under laboratory conditions and with pot trials. Resistance against phylloxera at roots and leaves was evaluated. Highest resistance degree was proved for variety *Börner*. Good resistance was found for most of tested rootstock varieties. Very good resistance have *SO4 PO 0/7* and *Craciunel 2 PO 0/6*. It is interesting, that hybrid *Kl×SO4* has also relatively good resistance. This promising franco-american needs further examination thanks to its good growing properties. After long time, this research offers new results about rootstock and phylloxera interactions under conditions of Southern Moravia. Results serve as a ground for further resistance breeding against phylloxera in Czech Republic.

Vitis vinifera, rootstock, grape phylloxera, damage, nodosity, leaf gall, resistance

Grape phylloxera, *Dactulosphaira vitifoliae* Fitch. (Homoptera: Phylloxeridae) was described in 1855 from native American *Vitis*. Currently a single species is recognized (Granett et al., 2001).

Grape phylloxera is regarded as the world´s worst grapevine pest. It is geographically widespread in most major grape growing regions of the world.

Rootstock use in viticulture is the consequence of phylloxera infestation during the second half of the 19 th century and is one of the first biological methods to control pests (Pouget, 1990).

In the middle of the 1990s a new grape phylloxera occurrence have been reported in European and American grape-growing areas (Remund, Boller, 1994; Omer et al., 1995).

In search of new rootstock/scion combinations, newly bred rootstocks are very helpful. The main selection criteria for new cultivars is their phylloxera tolerance (Becker et al., 2003).

Feeding of phylloxera on grapevine roots results in two types of galls. (1) Nodosities are galls formed on the tip of rootlets which are generally thought result in minimal damage to the vine. (2) Tuberosities are galls formed on larger, older portions of the root which, if sufficiently abundant and accompanied by microbiological pathogens, may eventually result in the death of the vine (El-Nady, Schröder; 2003).

A loss of growth of fresh roots, which is directly correlated with growth of leaves, is the consequence of feeding activity. High numbers of nodosities can lead to a reduced growth of leaf surface and thus to the previously mentioned damages (Porten et al., 2000).

Induction of leaf galls occurs on newly expanding leaves and once a leaf is fully expanded new galls cannot form (Rosen, 1916).

Resistance ratings of *Vitis* species have been relative consistent, although more recent studies have detected large variation in resistance among *Vitis* species accessions (Grzegorczyk, Walker; 1998).

The complex interactions of vine cultivar and physiology, environmental conditions, and phylloxera strain and physiology that limit gall formation have an unexplained mechanistic basis. Because gall formation is prerequisite to population survival, growth, and eventual vine damage, understanding these mechanism has immense practical significance (Granett et al., 2001).

Evaluating rootstocks for phylloxera resistance is a major hindrance to a breeding program (Walker, 1992).

Goal of this work are comparison and pot experiments with respect of resistance evaluation against phylloxera for ordinary rootstocks used in Czech viticulture. Resistance of these rootstock varieties to leaf phylloxera form was important also in rootstock nurseries.

MATERIAL AND METHODS

This experiment passed in the fields and laboratories in Faculty of Horticulture, Mendel University of Agriculture and Forestry, Lednice in 2004.

Evaluation was performed with rootstock varieties from grapevine gene resources. Evaluated varieties are shown in table III.

The phylloxera´s eggs originated from rootstock´s vineyard in the Grapevine breeding Station in Polešovice from leaf form on Kober 125 AA.

Laboratory bioassay

Roots 3–7 mm in diameter were washed with distilled water and cut into 5 cm sections. One end of each root piece was wrapped with moist cotton wool and the roots were placed in pairs on filter paper discs in 9x1 cm glass Petri dishes. We infested the roots with eggs of phylloxera from laboratory colonies. Colonies were maintained according to Granett et al. (1985) and De Benedictis, Granett (1992). We recorded the number of eggs, juvenile stages of phylloxera and grape phylloxera adults living on roots on days 18, 24 and 31 after inoculation.

Pot trial evaluation

Pot trial were realized in the 2 l volume pots. Rootstocks were propagated and rooted in hot greenhouse. Infection of leaf gallicoles were performed in June. Evaluation of resistence were performed in September. Leaf and root resistence were evaluated after succesive methodics.

Phylloxera gallicoles evaluation

We used the following indexing criteria (modified from Kozma et al., 1998) to evaluate sensitivity to gallicoles.

I: *Indexing criteria to evaluation of gallicoles (Kozma et al., 1998)*

Index	Symptoms on leaves	Sensitivity
1	No galls, occasional necrotic spots	none
3	Sterile galls, fewer than 15 small, fertile galls	low
5	More than 15 small, fertile galls	medium
7	Fewer than 20 large, fertile galls	high
9	More than 20 large, fertile galls	very high

Phylloxera nodosities evaluation

Adjusted Porten, Schmidt and Rühl (2000) degree scale was used for nodosities occurence evaluation at roots of observed varieties during pot experiments.

II: *Evaluation of roots in pot trial, Porten,Schmidt and Rühl (2000)*

Index	Symptoms
1 = no infestation	No nodosities and no phylloxera
3 = low infestation	Few new nodosities. Very low phylloxera populations. Only a few eggs.
5 = medium infestation	Several new nodosities, many old ones. Some phylloxera. Some eggs.
7 = heavy infestation	Many new and old nodosities. Many phylloxera, often several on one nodosity. Many eggs.
9 = extréme infestation	Lot sof new and old nodosities. Large numer of phylloxera and eggs. Usually several adults on one nodosity.

Experiment results were statistically evaluated by variance analysis ANOVA and followed by Tukey test at significance level $p < 0.05$.

RESULTS AND DISCUSSION

Testing a rootstock cultivar by planting it in the presence of a phylloxera population will show whether it has sufficient resistance to tolerate the specific biotypes of phylloxera that is represented by that population. When testing numerous rootstocks in a research breeding program, it becomes difficult to challenge all plant lines against an array of biotypes in field block. In the past, breeders overcame this problem by moving the tests into greenhouse for initial assay and then taking the best lines for further testing and evaluation (Granett et al., 1987).

Granett et al. (1987), indicate that laboratory evaluation can be used as a conservative predictor of resistance in the field.

Field trials and various greenhouse and laboratory studies relied upon as indicators of field performance of rootstocks (De Benedictis, Granett, 1993).

Table III. show laboratory experiment evaluation results – number of eggs at root fragments. Results suggest lower egg appearance at rootstock *Börner* a Teleki 5 C PO 3/7. On the contrary highest egg number was founded at *Amos* a *Fercal*. Results of ANOVA show high detectability of variety influence at phylloxera eggs occurence during laboratory experiment.

III: *Results of laboratory bioassay – evaluation of phyloxera´s eggs*

Rootstocks	Eggs		
	Day 18	**Day 24**	**Day 31**
Kober 5 BB	0.20a	0.20a	0.40ab
Teleki 5 C PO 3/7	0.60ab	0.00a	0.20a
SO 4 PO 0/7	1.20ab	0.20a	0.00a
Craciunel 2 PO 0/6	0.20a	0.00a	0.60ab
Kober 125 AA PO 0/3	1.00ab	0.60a	0.60ab
Amos	3.20b	3.60b	4.00c
K1 x SO 4	2.40ab	0.40a	0.80ab
Börner	0.80ab	0.40a	0.20a
Fercal	1.40ab	0.80a	1.80b
Riparia Portalis	0.00a	0.60a	0.40ab
F	**2.757**	**5.609**	**7.167**
Significance	**0.013**	**0.000**	**0.000**

Table IV. show phylloxera juvenile phases occurence at root fragments. Highest value was recorded with rootstock varieties *Amos* and *Fercal*. phylloxera occurence gradually decreased from day 18 till day 31st for all rootstock varieties. No phylloxera juvenile phase was found by *Börner* rootstock Results of statistical evaluation proved very high detectability of rootstock variety at juvenile phases occurence during all terms.

IV: *Results of laboratory bioassay – evaluation of phyloxera´s juvenile stages*

Rootstocks	Juvenile stages		
	Day 18	**Day 24**	**Day 31**
Kober 5 BB	0.40 a	1.00a	0.60ab
Teleki 5 C PO 3/7	0.40 a	0.40a	0.20ab
SO 4 PO 0/7	3.00 ab	0.40a	0.40a
Craciunel 2 PO 0/6	0.40 a	1.00a	0.60ab
Kober 125 AA PO 0/3	4.00 b	1.40ab	1.00ab
Amos	2.80 ab	3.40a	4.00c
K1 x SO 4	0.40 a	1.80ab	0.80ab
Börner	0.20 a	0.20a	0.00a
Fercal	1.00 ab	2.20ab	2.40bc
Riparia Portalis	0.60 ab	0.40a	0.20ab
F	**3.744**	**4.241**	**7.179**
Significance	**0.002**	**0.001**	**0.000**

Lowest phylloxera occurence was recorded during 31st day for every pure american grape species (*Riparia Portalis*) and hybrids of american species. Lowest phylloxera occurence was observed for rootstock *Kober 125 AA PO 0/3* and *K1 × SO4*. Higher phylloxera occurence had hybrids of american species with *Vitis vinifera – Amos* and *Fercal*.

V: *Results of laboratory bioassay – evaluation of phylloxera´s adults*

Rootstocks	Adults		
	Day 18	**Day 24**	**Day 31**
Kober 5 BB	0.20	0.40ab	0.00a
Teleki 5 C PO 3/7	0.00	0.40ab	0.00a
SO 4 PO 0/7	0.00	0.20a	0.00a
Craciunel 2 PO 0/6	0.20	0.40ab	0.00a
Kober 125 AA PO 0/3	0.00	2.60a	1.00ab
Amos	0.20	1.80ab	3.20a
K1 x SO 4	0.60	0.20a	0.80ab
Börner	0.00	0.00a	0.00a
Fercal	0.20	1.40ab	2.00ab
Riparia Portalis	0.00	0.20a	0.00a
F	**1.125**	**3.769**	**4.729**
Significance	**0.368**	**0.005**	**0.000**

Laboratory experiment results proved high resistance for rootstock Börner. Good resistance has *Kober 5 BB*, *Teleki 5 C PO 3/7*, *SO 4 PO 0/7*, *Craciunel 2 PO 0/6*, *Kober 125 AA PO 0/3*, *K1 × SO4* and *Riparia*

Portalis. Higher phylloxera occurence and reproduction was observed by *Amos* and *Fercal*. ANOVA test proved high detectability of variety influence at Phyloxera imago occurence only in day 31.

Interesting results from laboratory experiment discovered also (Kellow et al., 2002). On excised *V. vinifera* roots, nodosities were formed predominantly in the zone of root elongation, slighltly behing the root apex. On excised *Ramsey* roots, the single nodosity initiated was very close to the root tip. On excised *Schwarzmann* roots, a few crawlers settled near the root tips, but rapid browning of the root tissue occurred and crawlers died in situ. On excised *Börner* roots, crawlers preferentially settled at wound sites, for example those formed by detachment of lateral roots.

A bioassay using excised roots is especially useful for determining the influence of host variety or phylloxera strain on phylloxera life-table performance. From practical perspective, it is useful to optimize the bioassay conditions that allow the insect to exhibit performance near its intrinsic capacity for survival, development, and reproduction. Laboratory bioassays with different root variety-combinations suggest that there is a wide range in phylloxera adaptation to varieties (Granett et al., 2001).

Grape phylloxera is also able to feed on leaves with differences accord to the *Vitis* species (Wapshere and Helm, 1987; Williams and Shambaugh, 1988).

The level of phylloxera resistance of a candidate rootstock can be evaluated under field conditions (Nedov and Guler, 1974), in potted culture (Boubals, 1966) and using laboratory methods (Pouget, 1975).

American *Vitis* species show varying levels of resistance and tolerance to the phylloxera and a few accessions have been used to produce rootstocks for use in commercial viticulture (Pongrácz, 1983).

During pot experiment in 2004 grape phylloxera gallicoles occurence at leaves and roots were evaluated. Table VI. show phylloxera occurence at leaves and roots evaluation results. Lowest of phylloxera gallicoles leaf form was observed for rootstock resulted as hybrids between *Vitis* spp. x *Vitis vinifera – Amos*, *Fercal* and *K1 × SO4*. No leaf gallicoles were observed by *Börner* variety. *Börner* proved only strong defensive reaction resulting in necrotic spots (×ure 1), that prove its resistance to phylloxera. Higher phylloxera leaf form occurence was observed for rootstock originated from pure american *Vitis* species. ×ure 1 show necrotic reactions at leaf of *Börner* variety, whereupon ×ure 2 show gallicoles formation by *SO 4 PO 0/7*.

VI: *Evaluation results of Phyloxera occurence at leaves and roots during pot experiments*

Rootstocks	Evaluation of the leaf	Evaluation of the the root
Kober 5 BB	3.00bc	3.40 cde
Teleki 5 C PO 3/7	3.00bc	4.60 ef
SO 4 PO 0/7	4.60c	2.20 b
Craciunel 2 PO 0/6	2.60ab	2.60 bc
Kober 125 AA PO 0/3	2.20ab	4.20 def
Amos	2.60ab	5.00 f
K1 x SO 4	1.60ab	3.80 cdef
Börner	1.00a	1.00 a
Fercal	2.20ab	3.80 cdef
Riparia Portalis	2.20ab	3.00 bcd
F	**6.013**	**9.988**
Significance	**0.000**	**0.000**

Phylloxera occurence evaluation on leaves performed also Kozma et al. (1998). The leaf gall index was highest for the rootstocks *5 BB*, *5 C* and *Fercal* and intermediate for *101-14* and *Georgikon 28* and low for interspecific hybrids and the *Vitis vinifera* cultivars (Kozma et al., 1998).

The grape species also vary in the degree of their resistance to phylloxera, but the various description of this resistance are confusing. It is common for species to have strong resistance to root feeding, but poor resistance or no resistance to leaf feeding. The roots of most North American *Vitis* species resist the development of tuberosities and do not decline to root feeding. Yet many of these resistant species allow the development of nodosities on their root tips to varying degrees (Forneck et al., 2001).

Root system evaluation during pot experiment showed high statistically evidental variety influence at phylloxera occurence at roots. Highest resistance degree was proved for variety *Börner*. Rootstock breeders from Geisenheim found similar results. They use this variety for further breeding.

×ure 3 show root system of variety *Börner*, without nodosities, on the other hand ×ure 4 show nodosities occurence at roots of *AMOS*.

These results evaluated also Schmid et al., 2003. Some of seedlings, produced by Börner´s breeding work, were followed up by Helmut Becker and resulted in the release and classification of the variety *Börner*. Of course no rootstock can be suitable for all soil types and climatic conditions, therefore this resistance mechanism has to be combined with other viticultural characters. The current rootstock breeding program at Geisenheim aims for new rootstocks completely resistant to phylloxera, with a tolerance to virus transmission via nematodes, good rooting ability, high compatibility and good adaptation even to difficult soil types with a positive effect on grape and wine quality.

Good resistance was found for most of tested rootstock varieties. Very good resistance have *SO4 PO0/7* and *Craciunel 2 PO 0/6*. It is interesting, that hybrid *Kl×SO4* has also relatively good resistance. This promising franco-american needs further examination thanks to its good growing properties.

Good resistance to phylloxera for franco-american hybrids claim also Martinez-Peniche (1999), who wrote, that rootstocks with partial *Vitis vinifera* parentage may also confer a high level of grape phylloxera resistance, but this resistance is not durable.

Lowest resistance to Phyloxera was verified for multiple franco-american hybrid *Amos*, that is not very widespread in vineyards.

Correlation analysis showed interesting relation between evaluation under laboratory conditions after 31 days and phylloxera occurence at roots in pot experiment. Following correlational relations were discovered: eggs × pot trial – r = 0.51, juvenile stages × pot trial – r = 0.70, and adults × pot trial – r = 0.69.

1: *Defensive reaction resulted in necrotic spots in Börner*

3: *Root system of Börner without nodosities*

2: *Formation of gallicoles in SO4 PO 0/7*

4: *Nodosities occurence at roots of Amos*

SUMMARY

Grape phylloxera, *Dactulosphaira vitifoliae* Fitch is one of most destructive insect pest of cultivated grapes (*Vitis vinifera* L.) worldwide. Phylloxera was the cause of incomparable structural changes in European viticulture. It also led to the usage of new biotechnology – rootstocks for grape varieties.
Good resistance was found for most of tested rootstock varieties. Very good resistance have *SO4 PO0/7* and *Craciunel 2 PO 0/6*. It is interesting, that hybrid *Kl×SO4* has also relatively good resistance. This promising franco-american needs further examination thanks to its good growing properties.
After long time this research offer new knowledge about relations between rootstock and phylloxera under field conditions of Southern Moravia. These results can serve as pillow for further Czech breeding for resistance against phylloxera.

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SOUHRN

První zkušenosti se vzájemnými interakcemi podnož révy vinné x mšička révokaz

Mšička révokaz (*Dactulosphaira vitifoliae* Fitch) je jedním z nejvíce škodlivých škůdců u révy vinné (*Vitis vinifera* L.). Mšička révokaz způsobila velmi významné změny v evropském vinohradnictví. To také vedlo k použití nové biotechnologie – podnoží pro odrůdy révy vinné. Přímé poškození révokaze se projevuje na kořenech a listech. Podnože, které jsou vyšlechtěny výhradně z amerických *Vitis* spp., dovolují slabé nebo žádné poškození kořenů révokazem, na rozdíl od *Vitis vinifera*. Podnože byly hodnoceny v laboratorních podmínkách a v nádobovém pokusu. Odolnost proti révokazu na kořenech a listech byla hodnocena. Vynikající odolnost byla zjištěna u podnože *Börner*. Dobrá odolnost byla potom zjištěna u většiny sledovaných podnoží. Velmi dobrou odolnost mají podnože *SO4 PO 0/7* a *Craciunel 2 PO 0/6*. Je zajímavé, že kříženec *K1 × SO4* má také poměrně vysokou odolnost. Tento perspektivní franko-amerikán vyžaduje další zkoušení díky jeho dobrým pěstitelským vlastnostem. Po dlouhé době tento výzkum poskytuje nové výsledky o vzájemném působení podnože a révokaze v podmínkách jižní Moravy. Výsledky slouží jako základ pro další šlechtění na odolnost k révokazu v České republice.

réva vinná, podnož, mšička révokaz, napadení, nodosity, listové hálky, rezistence

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