# Resistance of rootstocks to the virus transmitting nematode *Xiphinema index.*

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## Abstract

Virus transmission was studied on rootstock varieties and *Vitis* species to identify suitable genotypes for breeding purposes. Apart from *Vitis rotundifolia* also *Vitis cinerea* Arnold showed a high degree of resistance. As *V. rotundifolia* is difficult to use in breeding program, *V. cinerea* is a species to consider, if high phylloxera and virus transmission resistance are required in combination with good rooting and high affinity. The new rootstock Börner proves this point.

## Introduction

Virus diseases are a serious problem in grape growing areas around the world. The nepo viruses of the fanleaf complex, e.g. GFLV, ArMV, RRV, SLRV are transmitted by nematodes of the *Xiphinema* and *Longidorus* group. *Xiphinema index*, the vector of GFLV is the most important species of them and common in most traditional wine growing regions of Europe.

The threat of fanleaf disease, transmitted by *X. index*, is increasing, due to restricted or forbidden soil fumigation in many countries. Therefore alternative ways to fight virus transmission via *X. index* are required. A biological control mechanism would be an elegant solution, in particular, if it could be achieved by resistant rootstocks.

Resistance of the species *Vitis rotundifolia* against virus transmission has been reported several times (Staudt and Weischer, 1992; Bouquet 1981). But this variety is difficult to work with in breeding programs, due to different chromosom numbers. Carl Börner (1934) detected in *Vitis cinerea* Arnold a phylloxera resistance as high as that of *Vitis rotundifolia*. Becker (1989) assumed the resistance against phylloxera and nematodes to originate in a similar physiological mechanism. *V. cinerea* therefore might also have at least some resistance against virus transmission. The aim of this study was to investigate the nematode resistance and the degree of virus transmission of rootstock varieties and *Vitis* species with a particular focus on *V. cinerea* and its hybrids.

## Material and Methods

A series of virus transmission studies with *X. index* were conducted in a greenhouse with rooted cuttings of 5C Geisenheim clone 6-13 Gm, Kober 5BB clone 13 Gm, Rupestris du Lot (Rupestris St. George), *Vitis cinerea* Arnold, *Vitis rotundifolia*, and two *V. riparia* 183 G x *V. cinerea* Arnold hybrids: 'Seeliger' and ' Börner'.

Experiments were carried out in 8 cm ceramic pots at 20 to 25 °C air temperature. The nematodes were pre-multiplicated on *Ficus carica* L and, in order to take up GFLV, were then cultured for 4 months on fanleaf infected vines. To each pot 50 adult *X. index* were added, either infected or non-infected with GFLV Control vines were grown without nematodes. Usually 5 replicates were used.

After 12 months trials were terminated: Nematode numbers were recorded and the virus status of the plants analyzed by ELISA. The wound reaction of roots after nematode attack was studied by light-microscopy after sample fixation and imbedding in 2-Hydroxyethylmethacrylat (Karnovsky, 1965). Sample thickness was 1 to 2 µm and staining carried out by a 0.1 % Toluidinblue solution. In addition on nematode attacked and non-attacked roots peroxidase and polyphenoloxidase activity were measured (Sopp, 1994).

## Results and Discussion

### Nematode development on different hosts

Nematode population at the end of the first trial was generally higher, than of the second one (Fig. 1). In both trials nematode numbers were significantly affected by the genotype of the host plant. It was highest in 5C pots with nematode numbers increasing by 1200 % and 325 %, respectively. *V. cinerea* and *V. rotundifolia* were only included in trial 1, but those species were the only host plants in the trial, which reduced nematode numbers during the experiment by 6 and 72 %, respectively. Among the rootstock varieties in the trials Börner had the lowest increase in nematode numbers (273 % and 124 %), indicating its poor host qualities for *X. index* and its ability to keep nematode numbers low.

### Histological studies on roots after *X. index* attack

On roots typical changes were observed after nematode attack: On 5C, 5BB and Rupestris du Lot characteristic root galls developed, while *V. rotundifolia*, *V. cinerea* and descendant of *V. cinerea* e.g. Seeliger and Börner showed no root galls. On places of nematode attack they produced necrotic lesions. Histological examination of root tips attacked by *X. index* showed giant cells with multiple, irregularly shaped nuclei. On *V. rotundifolia*, *V. cinerea*, Börner and Seeliger on places of earlier nematode attack no galls and no giant cells with multiple nuclei were found. Insted those genotypes showed necrotic spots, consisting of suberised and collapsed cells, characteristic for hypersensitive wound reactions.

It can therefore by concluded that the suitability as host of *Vitis* genotypes is different. While 5C appears to by a good host, leading to high increases in nematode numbers, the species *V. cinerea* Arnold and *V. rotundifolia* were poor hosts, actually reducing population numbers. Börner was the rootstock with the poorest host qualities.

### Peroxidase and polyphenoloxidase activities after *X. index* attack

Peroxidase (PO) and polyphenoloxidase (PPO) activities were increased in roots after nematode attack (Fig. 2). With no nematodes present all genotypes in the trial had similar PO and PPO activities, except PO activity of *V. cinerea.* Enzym activies in all varieties increased, when nematodes where present in the pots. Highest activities were found in roots of *V. cinerea*, Seeliger and Börner. It can therefore be assumed that increased PO and PPO activities after nematode attack are a reaction of the plant against the attack. Therefore the poor host quality of *V. cinerea*, Seeliger and Börner may also be a result of their higher PO and/or PPO activities.

**Virus transmission by *X. index***

Significant treatment differences could also be found in regards to virus transmission (Table 1). While no GFLV could be found in control vines and in vines treated with non-infected *X. index*, transmissions occurred when GFLV carrying nematodes had been added to the pots. In the first experiment one vine each of five 5BB and Rupestris vines and three of the five 5C became infected with GFLV during the trial, but none of the *V. cinerea* Arnold, *V. rotundifolia*, Seeliger and Börner vines. In the second trial even all 5C and 5BB plants and four of five Rupestris du Lot plants got infected. *V. cinerea* Arnold and *V. rotundifolia* were not included in the trial, but again no infection could be detected in any of the Börner and Seeliger vines.

## Conclusion

It can therefore be assumed that *V. cinerea* Arnold has apart from a complete phylloxera resistance (Becker, 1990) also a high degree of resistance against GFLV transmission via *Xiphinema index* and that this resistance has been successfully transmitted to some of its hybrids, e.g. Seeliger and Börner. This not only provides the viticultural industry with an interesting new rootstock, it also indicates that virus transmission resistance can not only be achieved with *V. rotundifolia*, but also with *V. cinerea* Arnold. Due to no genetical barriers between *V. cinerea* and other species commonly used in rootstock breeding, *V. cinerea* Arnold should be more considered as parent in breeding programs.

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Table 1: GFLV transmission by the nematode *X. index* after 12 month of inoculation.
*V. cinerea* and *V. rotundifolia* were not included in trial 2.
Numerator = number of infected vines; denominator = total number of vines.

| treatment | genotyp | number of vines with GFLV transmission detected trial 1 | number of vines with GFLV transmission detected trial 2 |
|---|---|---|---|
| control vines without nematodes | 5C | 0 / 5 | 0 / 5 |
| | 5BB | 0 / 5 | 0 / 5 |
| | Rupestris du Lot | 0 / 5 | 0 / 5 |
| | Seeliger | 0 / 5 | 0 / 5 |
| | Börner | 0 / 5 | 0 / 5 |
| | *V. cinerea* Arnold | 0 / 5 | - |
| | *V. rotundifolia* | 0 / 5 | - |
| addition of *Xiphinema index* not infected with GFV | 5C | 0 / 5 | 0 / 5 |
| | 5BB | 0 / 5 | 0 / 5 |
| | Rupestris du Lot | 0 / 5 | 0 / 5 |
| | Seeliger | 0 / 5 | 0 / 5 |
| | Börner | 0 / 5 | 0 / 5 |
| | *V. cinerea* Arnold | 0 / 5 | - |
| | *V. rotundifolia* | 0 / 5 | - |
| addition of *Xiphinema index* infected with GFV | 5C | 3 / 5 | 5 / 5 |
| | 5BB | 1 / 5 | 3 / 3 |
| | Rupestris du Lot | 1 / 5 | 4 / 5 |
| | Seeliger | 0 / 5 | 0 / 5 |
| | Börner | 0 / 5 | 0 / 5 |
| | *V. cinerea* Arnold | 0 / 5 | - |
| | *V. rotundifolia* | 0 / 5 | - |