Resistance to transmission of grapevine fanleaf virus by *Xiphinema index* in some *Vitis* species and hybrids

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S u m m a r y : Many vineyards in Germany are infested by nematodes which can transmit virus diseases. Breeding of rootstocks resistant to nematode feeding and virus transmission is an important way to control these virus diseases.

A method has been developed for testing breeding stocks for these characteristics with regard to grapevine fanleaf virus (GFV) and its vector *Xiphinema index*.

The plants to be tested were first grown together in a single pot with both GFV-infected plants of *Vitis* cv. Siegfried and plants of the same cultivar which were virus negative according to an ELISA test: 2 months after planting, the pots were inoculated with about 50 nematodes. In a second experiment, single healthy plants of different hybrids and species were potted and inoculated with about 200 nematodes carrying GFV. After 3-10 months, roots were inspected visually for swellings and galls and tested for the presence of GFV by ELISA.

In all cases, the previously healthy cv. Siegfried showed symptoms of feeding on the roots and these roots showed a positive reaction to the ELISA test. After 6 months, GFV could also be detected by ELISA in the basal parts of the stems.

The reaction of the test plants was dependent on their genotype. A high susceptibility to feeding by the nematodes and high percentage of transmission to GFV was displayed by American species and interspecific hybrid rootstocks. One of the *V. vinifera x V. rotundifolia* hybrids showed no visual symptoms of nematode feeding and no virus transmission 9 months after inoculation.

K e y w o r d s : Vitis, variety of vine, root, fanleaf, virus, nematodes, Xiphinema index, vector, transmission, resistance, bioassay.

Introduction

Many vineyards in Germany are infested by nematodes which can transmit viruses. The most serious viral pathogens are:

grapevine fanleaf virus (GFV)
arabis mosaic virus (ArMV)
raspberry ringspot virus (RRV)
tomato blackring virus (ToBRV)
strawberry latent ringspot virus (SLRV)

These so-called nepoviruses are transmitted by different species of nematodes, of which *Xiphinema index* is perhaps the most important one due to its known ability to transmit GFV and to its worldwide distribution. It is widely accepted that ArMV is vectored by *X. diversicaudatum*, raspberry ringspot virus by *Longidorus macrosoma*, and tomato blackring virus by *L. attenuatus*.

For many years German grape growers were quite successful in controlling virus diseases transmitted by nematodes with pre-plant soil fumigation. However, in recent years efficient nematicides have become unavailable. Breeding rootstocks resistant to nematodes now appears to be the sole solution to the problem of nematode transmitted virus diseases.

As pointed out by KUNDE *et al.* (1968) and in accordance with ROHDE (1965), COOK and EVANS (1987) and MÜLLER (1989), resistance against nematodes can be defined as an interaction between the nematodes and grapevines which retards or prevents maturation and/or reproduction of the nematodes. Damage of feeding of the parasitic nematodes is not the problem in Germany, but rather virus transmission. Therefore, only an extreme of resistance, which could be called absolute or high resistance, is the type of resistance we are looking for. Some authors, for example HARRIS (1983), used the term immunity for this type of resistance. But, as immunity is widely used with describing antigen-antibody reactions, this term should be avoided in plant pathology.

The production of swellings and/or galls is a reaction of the host plant and therefore a question of sensitivity to the attack of the nematodes. Differences occur with respect to this characteristic between species and cultivars, but this reaction should not be regarded as a type of resistance. Plants which show no reaction or decline in yield are called tolerant. As pointed out earlier, we are not interested in tolerant plants because virus transmission is the paramount problem and this can only be solved satisfactorially by absolutely resistant rootstocks or such plants which do not allow virus transmission.

Any breeding project should begin with screening the collections for resistance. In regard to resistance against the dagger nematode *X. index*, this has already been done bgy various authors such as KUNDE *et al.* (1968), BOUBALS and PISTRE (1978), BOUQUET (1981) and COIRO *et al.* (1985). In regard to transmission of viruses the results are not always convincing. KUNDE *et al.* rated *V. arizonica* and *V. candicans*, in addition to other species, as resistant. According to the investigations of WEISCHER (1980) these are only tolerant. This has been confirmed by our recent investigations which showed that GFV could easily be transmitted to *V. arizonica* by *X. index.*

Materials and methods

Tests for nematode resistance with absolute resistance in view should only be conducted under controlled application of nematodes, as described by BOUBALS and PISTRE (1978) and BOUQUET (1981). The goal of our investigations was to develop a method with which plants could be screened for nematode resistance and/or virus transmission within a reasonable time.

All plants tested were *in vitro* propagated and therefore absolutely free of nematodes and GFV. In our initial experiments, a plant to be tested was grown together in a single pot with a GFV infected plant of cv. Siegfried, which is an interspecific hybrid very sensitive to nematode feeding and to which it is very easy to transmit GFV. Another plant of the cv. Siegfried, which was virus negative according to an ELISA test, was planted in the same pot. 2 months after planting, the pots were inoculated with 20 ml soil containing about 50 nematodes. Occurrence of nematode feeding and subsequent transmission of virus was monitored by the virus negative Siegfried plant.

In our further experiments the pots with single plants to be tested were inoculated directly with ca. 200 viruliferous (GFV) nematodes. After different lengths of time, roots were inspected visually for swellings and/or galls and tested for the presence of GFV by ELISA.

Results and discussion

Already 3 months after inoculation it was possible to make definite statements about the host reaction and virus transmission as well. This was, for example, the case for *V. rupestris, V. riparia* and the rootstocks Kober 5 BB and 125 AA. There were certain genotypes which needed further investigation or needed a longer exposure to nematode feeding to assure an accurate rating.

In the table results are summarized which were collected over a period of 3-10 months of exposure to nematodes. A high susceptibility to feeding by *X. index* and high percentage of transmission of GFV was displayed by:

the rootstocks:

cv. Kober 5 BB *(V. riparia x V. berlandieri)*

cv. Kober 125 AA *(V. riparia x V. berlandieri)*

cv. FR 419 a newly released cultivar with *V. cinerea* in its pedigree

the interspecific hybrids:

cv. Siegfried

cv. FR 993-60 one of the most promising selections for wine production (STAUDT *et al.* 1984) and

Transmission of GFV by *Xiphinema index* to *Vitis* species and cultivars within 3-10 months

Species/cultivar	Number of plants tested	Number of GFV infected plants	% Infection	Symptoms of roots to nematode feeding
cv. Kober 125AA	26	26	100	+
cv. Kober 5BB	26	26	100	+
cv. FR 419	16	16	100	+
cv. Siegfried	120	95	79	++
cv. FR 993-60	53	45	85	++
V. arizonica	15	9	60	-
V. riparia gloire	24	22	92	+
V. rupestris	37	35	95	++
No. 030-51	110	64	58	+
cv. Riesling	26	9	35	++
No. 043-43	62	10	16	+
No. 039-16	60	0	0	-

the species:

V. arizonica
V. riparia
V. rupestris
No. 030-51 which is identical with *V. vinifera* #4 from the Middle East (WALKER *et al.* 1985).

There seems to be a reasonable resistance to virus transmission in cv. Riesling and this is being further investigated.

As expected, the two *V. vinifera x V. rotundifolia* hybrids obtained by OLMO (1954) (PATEL and OLMO, 1955; JELENKOVIC and OLMO 1968) showed the best resistance ratings. Only 10 out of the 62 plants tested of No. 043-43 showed virus transmission as a result of nematode feeding. This result, similar to that of cv. Riesling, but to a lesser extent, is as yet unexplained. It may be accounted for by a reduced attraction of nematodes by the roots, or to a reduced transmission or replication of the viruses.

The highly resistant No. 039-16 showed visually no symptoms of nematode feeding at all. Up to now, we do not know whether this really can be attributed to prevention of nematode feeding, virus transmission or virus replication.

Our investigations under way are in favor of the first explanation. According to recent results of WEISCHER (1988), the failure of virus transmission to *V. rotundifolia* by *X. index* may be attributed to a sensitivity reaction which prevents virus replication and/or virus distribution. This would mean that we can reckon with sources of resistance against nematode feeding and virus transmission in *V. rotundifolia* and their hybrids.

Both hybrids are already patented by the University of California Davis and recommended for planting (WALKER *et al.* 1989). Unfortunately, these hybrids cannot be used as rootstocks in Germany. Poor adaptation to our climatic conditions is the main handicap. But there are also some difficulties in using No. 039-16 in our breeding program. Cytological disharmonies in this hybrid, which may result from the different chromosome numbers of the parent species, lead to a serious reduction in fertility. Pollen fertility of No. 039-16 is below 1 % and the pollen grains, which seem to be functional, are giant pollen grains which may have originated by restitution during meiosis. As a consequence, they may have the doubled somatic chromosome number.

From the investigations conducted to date it can be concluded that resistance to nematode feeding and virus transmission is a rare characteristic and screening relevant species will be necessary to search for further sources of resistance.

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Detection of arabis mosaic virus and grapevine leafroll virus I during the period of vegetation

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A b s t r a c t : In 1987, at 13 different dates leaf samples from vines infected with AMV and GLRVI were collected from different positions in the plants.

All results showed that the Tris extraction buffer system produced better results than the Nicotin buffer system.

Before flowering AMV could be detected in young leaves better than in elder ones with all buffer systems, whereas after flowering the results changed and it was possible to detect AMV only with the Tris system in elder leaves.

For GLRVI only the Tris buffer system was used. GLRVI could only be detected from the end of August to the end of the vegetation period.

Samples from elder leaves produced higher extinction than those from younger ones for GLRVI.

A correlation between the results in ELISA test and the temperatures before the sampling dates could not be proved.