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Selection of mild virus strains of fanleaf degeneration by comparative field performance of infected grapevines

by

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Summary: Healthy clones of *Vitis vinifera* L. cultivars Klevener de Heiligenstein, Chardonnay and Pinot noir were graft-inoculated with one clone of the rootstock Kober 5BB infected with potential mild strains of arabis mosaic virus (ArMV-Ta) or grapevine fanleaf virus (GFLV-CB844 or -F13). Such infected vines were planted in a nematode-free replant site and screened for comparative field performance on pruning weight, crop yield, bunch weight, and sugar content over a 5-year period. ArMV-Ta had the mildest impact on both vigor and yield for all three *V. vinifera* cultivars tested. The impact of ArMV-Ta, along with the other two GFLV strains, was much less evident during the last two years of the trial. Based on these results, ArMV-Ta was selected as a potential mild strain for cross-protection to control fanleaf degeneration. Our field trial also showed that field performance of infected vines was not affected by the vein mosaic virus-like disease.

Key words: fanleaf degeneration, GFLV, ArMV, grapevine infection, field performance analysis, mild strain, cross-protection.

Introduction

Fanleaf degeneration is a severe disease of grapevine that occurs worldwide and is caused by nepoviruses. Vigor and yield of infected vines are affected and their longevity is reduced (BOVEY *et al.* 1980; PEARSON and GOHEEN 1988). Grapevine fanleaf virus (GFLV) is the principal causal agent of the disease, although other nepoviruses such as arabis mosaic virus (ArMV), tomato black ring virus, raspberry ringspot virus, strawberry latent ringspot virus, grapevine Hungarian chrome mosaic virus, artichoke Italian latent virus, grapevine Bulgarian latent virus, are also detected in degenerated cultivars in Europe.

In France, GFLV and ArMV, two serologically distinct nepoviruses (WALTER *et al.* 1984), are both responsible for fanleaf degeneration. The grape to grape transmission is specifically assumed by the *Xiphinema* longidorid nematode, *X. index* for GFLV and *X. diversicaudatum* for ArMV (BOVEY *et al.* 1980). Control of the natural spread is difficult because the nematode vectors are resistant to eradication by soil treatments with nematicides and/or fallow. Also, nematicides are expensive, not efficient in deep soils (RASKI *et al.* 1983), and environmentally harmful. Therefore, new control strategies have to be developed.

Cross-protection, the phenomenon by which a mild strain of a virus can protect a plant against detrimental effects against infection by a severe related strain, is a possible control measure for fanleaf degeneration (VUITTENEZ *et al.* 1978). The effectiveness of this approach was previously analysed on *Chenopodium quinoa* with several combinations of ArMV and GFLV strains. Plants inoculated with the mild ArMV-S strain were protected against challenge inoculations by the severe GFLV-F13 strain (HUSS *et al.* 1989).

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However, the selection of protecting strains that are mild on grapevine is an essential primary step in the use of cross-protection to control fanleaf degeneration in grapevines. In this study, we analysed two GFLV or one ArMV strains selected for the variability of symptoms they induced on *C. quinoa* and compared their effect on field performance of several grapevines. Our results indicate that ArMV-Ta is a good candidate that might be used as potential protecting strain in cross-protection experiments.

Materials and methods

Virus strains: ArMV (-S, -Ta) and GFLV (-CB844, -F13) strains were isolated from field infected *Vitis vinifera* L. cultivars Syrah, Tannat, Cabernet franc (VUITTENEZ *et al.* 1964) and Muscat (BOUBALS 1962). All strains were mechanically transmitted to several herbaceous host plants and maintained in the greenhouse on *C. quinoa* by periodic transfers through mechanical inoculations.

Grapevine infection: Clonal material was used in order to investigate the specific influence of different viral strains on the severity of fanleaf degeneration on the following *V. vinifera* cultivars: Chardonnay (clones 52C9 and 52C8), Klevener de Heiligenstein (clones TO 1165 and TG 65), a local Gewürztraminer non-aromatic Savagnin rosé cultivar, and Pinot noir (clone TO 1557). The rootstock used was *V. berlandieri* × *V. riparia* Kober 5BB (clone 259). Test plants originated from cuttings treated by heat therapy (BASS and LEGIN 1981) and indexed on woody indicators to detect known viruses and virus-like diseases.

Test plants were inoculated by the *in vitro* heterografting technique (BASS and VUITTENEZ 1979). Virus transmission to the inoculated vines was confirmed by serological indexing using polyclonal antibodies specific for ArMV or GFLV (ETIENNE *et al.* 1991) and leaves, rootlets or wood shavings as antigen source.

Field trial: A block of 190 vines was established in April 1984 in land not previously planted with grapevines and treated with D-D (1,3-dichloropropene and 1,2-dichloropropane at 100 l/ha). After soil treatment, a 3-year fallow was practiced. *Xiphinema* nematodes were not found in soil samples analysed according to the procedure described by SEINHORST (1955).

A randomized block design with 2—5 replicates of 5-vine plots was used. The vines were trained according to a system called 2Mb, which is characterized by a vertical single plane trellis maintained with 3 wires (SCHNEIDER *et al.* 1989). The vines were spaced 1 m apart with 2 m between rows. Healthy vines were planted at the ends of each trial row and on each side in order to completely surround the trial block. The vines were pruned according to visual assessment of vigor (2 canes were retained for each vine) by technicians who were not informed about the sanitation status of the planted vines to prevent bias in the training.

Field performance analyses: Annual measurements were taken of pruning weight, fresh weight of grapes, number of bunches and sugar content of berries from 1986 to 1990. Each vine was individually analysed for these 4 factors. Total sugar content was determined in juice extracted from all grapes collected from one replicate of 5 vines immediately after harvest. The mean bunch weight was calculated from the data obtained.

Mean values for each parameter were calculated for each combination of *V. vinifera*-virus strain both per year and over 5 years. Data were subjected to statistical analysis and means were compared using the least significant difference at the 0.05 probability level with Student's t-distribution test.

Results

Selection of potential mild virus strains causing fan-leaf degeneration: Several strains of ArMV and GFLV were initially collected from infected grapevines showing a range of symptoms. Typical leaf symptoms were mild to severe malformations, yellow mosaic, vein banding, and chlorotic spots. Strains inducing mild symptoms were selected among other more detrimental strains and further screened for the symptoms induced in *C. quinoa*. GFLV-F13 caused chlorotic spots on inoculated leaves followed by a vein clearing and a strong persistent mosaic with deformations of the apical *C. quinoa* leaves (VUITTENEZ *et al.* 1964), whereas GFLV-CB844 induced a weak non persistant vein clearing. ArMV-Ta and ArMV-S were responsible for a mild discrete mosaic (HUSS 1986).

Effect of viral strains on field performance of *V. vinifera* varieties grafted on Kober 5BB rootstock: The comparative field performance was evaluated over 5 years in order to avoid misleading interpretations due to fluctuations depending on poor growing seasons.

Most infected vines showed significantly less weight of pruning wood than healthy control and differences were observed between the effect of the three viral strains (Fig. 1, A). ArMV-Ta, GFLV-CB844 and GFLV-F13 induced 8, 51 and 14 % losses, respectively on infected Klevener de Heiligenstein. Values for ArMV-Ta were not significantly different at $P \leq 0.05$ from the healthy vines, however, GFLV-CB844 and GFLV-F13 scored with a significant difference from the control and ArMV infected

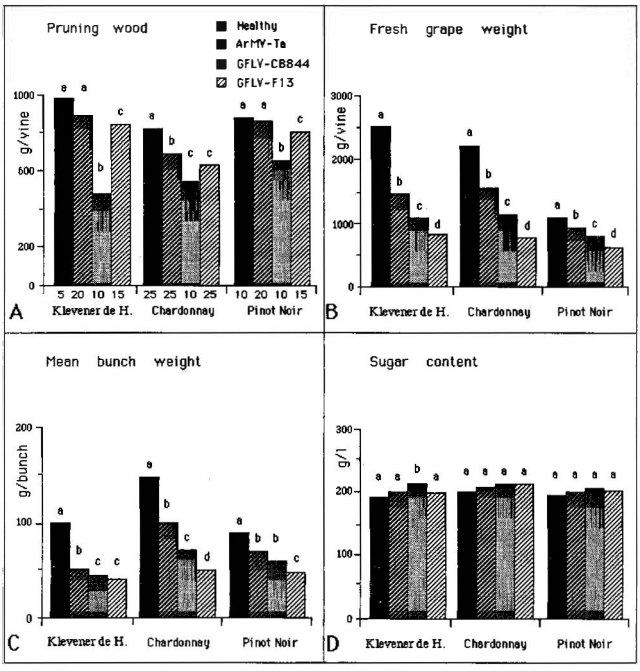

Fig. 1: Comparative field performance from 1986 to 1990 of *V. vinifera* Klevener de Heiligenstein, Chardonnay and Pinot noir grafted on Kober 5BB rootstock. Means accumulated over 5 years for pruning wood weight (A), fresh weight of grapes (B), mean bunch weight (C) and sugar content of berries (D) were analysed for healthy (■) or ArMV-Ta, GFLV-CB844 and GFLV-F13 infected vines, respectively. Number of vines tested per treatment for each combination is indicated below the X-axis. Letters indicate significant differences at $P \leq 0.05$ within cultivars.

vines. For Chardonnay, the same sequence of viral strains showed 17, 33, and 22 % decreases, respectively. These values were significantly different at $P \leq 0.05$ from the healthy vines and the values for GFLV-CB844 and GFLV-F13 were significantly different from ArMV-Ta. Infected Pinot noir was the least affected variety with only 1, 26 and 8 % losses. In that case, ArMV-Ta infected vines performed equally well to the healthy controls.

Considering the production of grapes (Fig. 1, B), infected vines yielded much less than controls and there was a differential decrease according to the virus strain/cultivar combination. ArMV-Ta, GFLV-CB844 and GFLV-F13 induced 43, 57 and 68 % yield losses, respectively for Klevener de Heiligenstein. Infected Chardonnay showed 30, 48 and 64 % decreases with the same viral strains, whereas only 15, 28 and 45 % of losses were recorded for infected Pinot noir. All the values for infected vines were significantly different from the healthy control vines and also between the 3 viral strains.

The number of bunches estimated for each vine (results not shown) allowed us to calculate a mean bunch weight for the healthy and diseased vines (Fig. 1, C). Significant differences were observed between healthy and infected *V. vinifera* and between the 3 viral strains tested. Klevener de Heiligenstein showed 48, 57 and 60 % decreases for ArMV-Ta, GFLV-CB844 and GFLV-F13 infected vines, respectively. The same sequence of viral strains induced 31, 40 and 68 % of losses, respectively on infected Chardonnay whereas only 22, 26 and 45 % losses were recorded for infected Pinot noir. Values for infected vines were significantly different from the controls and differences between viral strains were also detected in some cases.

Considering the sugar content of berries (Fig. 1, D) and, thus, the expected alcohol content of the wine produced, all three infected cultivars showed a slight increase of 1—6 % when compared to corresponding healthy vines. However, the differences recorded were not significantly different from the control vines and between viral strains, except for GFLV-F13 infected Klevener de Heiligenstein.

Analysis of performance over time: The mean weight of pruning wood of Chardonnay vines (clone 52C9) infected with the 3 viral strains was recorded each year from 1986 to 1990 (Fig. 2). The results obtained clearly showed an evolution of the weight of pruning wood over time. The differences between infected vines and healthy controls were wider during the two first years than during the latest years, indicating that the relative performance changed as the infected vines matured. Similar observations were realized with infected Pinot noir and Klevener de Heiligenstein. An overall identical regular decrease of the impact of the viral strains was also observed for the mean bunch weight. However, since grape is a long term crop, the differences observed the two first years may not be significant over time.

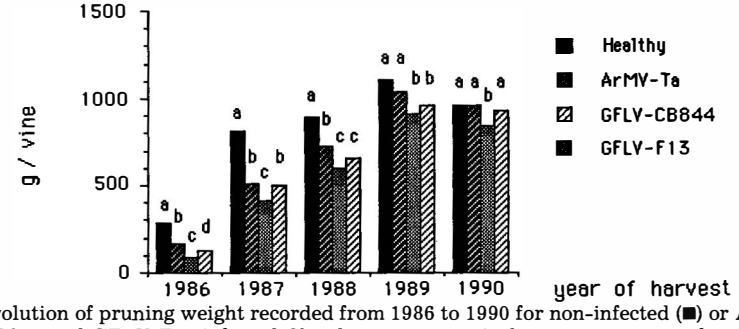

Fig. 2: Evolution of pruning weight recorded from 1986 to 1990 for non-infected (■) or ArMV-Ta, GFLV-CB844 and GFLV-F13 infected Chardonnay, respectively. Letters on top of each column indicate significant differences at $P \leq 0.05$ within years.

Effect of virus strains on different clones of Chardonnay: In order to control fanleaf degeneration by cross-protection, it was essential to verify if the results we observed were reproducible with other clones of *V. vinifera*. Therefore, the performance of 2 clones of Chardonnay (52C9 and 52C8) infected with 2 different GFLV strains (-CB844 and -F13) were recorded and compared (Tab. 1). No significant major difference in losses at $P \leq 0.05$ was observed between the 2 infected clones.

Table 1. Comparative field performance of Chardonnay clones 52C9 and 52C8 grafted on Kober 5BB rootstock infected with GFLV-CB844 or GFLV-F13

		Pruning wood (g/vine)		Fresh grape weight (g/vine)		Mean bunch weight (g/bunch)		Sugar content (g/l)	
Vitis vinifera	Kober 5BB rootstock		% loss		% loss		% loss		% increase
Chardonnay 52C9	non-infected (25) *	647 ± 137		5822 ± 664		609 ± 130		205 ± 4	
	GFLV-CB844 (25)	366 ± 123	**43** a**	2582 ± 449	**56** a	283 ± 10	**54** a	211 ± 4	**+3** a
	GFLV-F13 (15)	427 ± 119	**34** b	1549 ± 523	**66** b	201 ± 37	**67** b	211 ± 4	**+3** a
Chardonnay 52C8	non-infected (10)	836 ± 153		3995 ± 407		530 ± 57		209 ± 3	
	GFLV-CB844 (10)	501 ± 119	**40** a	1778 ± 255	**55** a	257 ± 42	**51** a	215 ± 4	**+3** a
	GFLV-F13 (20)	612 ± 139	**27** c	1614 ± 236	**60** b	205 ± 36	**61** b	214 ± 7	**+3** a

* Number of vines analysed

** Values with different letters are significantly different at $P \leq 0.05$

Effect of vein mosaic virus-like disease on field performance of two varieties grafted on Kober 5BB rootstock: After heat-treatment of the Kober 5BB mother plants, two sanitary families were regenerated and identified by indexing. The first family was completely virus-free and the second family remained infected with the vein mosaic virus-like disease.

In order to analyse the impact of the vein mosaic disease, we compared the field performance of two clones of Chardonnay and one clone of Klevener de Heiligenstein grafted on either healthy or on vein mosaic diseased rootstocks (Tab. 2). Since all horticultural parameters recorded were significantly identical at $P \leq 0.05$, no difference was noticed between the non-infected and the vein mosaic infected vines.

Furthermore, the impact of the vein mosaic disease was analysed with fanleaf diseased vines in order to check a possible synergy between fanleaf degeneration and vein mosaic (Tab. 3). Analysis of the different horticultural parameters indicated no significant effect at $P \leq 0.05$ between the 2 groups of vines.

Discussion

The results reported in this communication show that it is possible to select mild virus strains of fanleaf degeneration by comparing the field performance of infected grapevines. From the 3 strains tested, ArMV-Ta showed a promising potential as a mild protecting strain for cross-protection because it had a mild impact on both vigor and yield of the 3 *V. vinifera* Chardonnay, Pinot noir and Klevener de Heiligenstein, while GFLV-CB844 and GFLV-F13 caused more severe damage (Fig. 1).

Table 2. Field performance comparison of Chardonnay (clones 52C8 and 52C9) and Klevener de Heiligenstein (clone TG65) grafted either on healthy or on vein mosaic diseased Kober 5BB rootstocks

Vitis vinifera	Kober 5BB rootstock	Pruning wood (g/vine) *	Fresh grape weight (g/vine)	Bunch number (No/vine)
Chardonnay 52C9	non-infected	822 ± 206 **	3954 ± 554	16 ± 5
	vein mosaic infected	820 ± 151	4203 ± 795	17 ± 4
Chardonnay 52C8	non-infected	834 ± 131	4041 ± 444	19 ± 7
	vein mosaic infected	839 ±175	4181 ± 624	19 ± 5
Klevener de Heil.	non-infected	802 ± 200	5967 ± 488	32 ± 6
	vein mosaic infected	817 ± 128	6282 ± 577	30 ± 5

* Weight of pruning wood, fresh weight of grapes and number of bunches were recorded from 1986 to 1990 for Chardonnay clone 52C9, from 1988 to 1990 for Chardonnay clone 52C8 and from 1989 to 1990 for Klevener de Heiligenstein clone TG65.

** Means are indicated with the standard deviation. Values for non-infected and vein mosaic infected vines within cultivars were not significantly different at $P \leq 0.05$.

Table 3. Field performance comparison of Chardonnay (clone 52C8) grafted either on a Kober 5BB rootstock inoculated with GFLV or ArMV or on a vein mosaic diseased Kober 5BB rootstock inoculated with GFLV or ArMV

Virus strain	Kober 5BB rootstock	Pruning wood (g/vine)	Fresh grape weight (g/vine)	Bunch number (No/vine)
GFLV-F13 *	non-infected	594 ± 121	1560 ± 192	17 ± 5
	vein mosaic infected	630 ± 157	1665 ±267	16 ± 4
ArMV-S	non-infected	695 ± 150	1231 ± 586	19 ± 8
	vein mosaic infected	694 ± 121	1287 ± 207	20 ± 6

* Horticultural parameters were recorded over 3 years for the GFLV-F13 infected vines and over 2 years for the ArMV-S infected vines. Values for non-infected and vein mosaic infected vines within treatments were not significantly different at $P \leq 0.05$.

Grape is a long term crop for which important yield occur 4—5 years after planting. Hence, potential protecting mild viral strains selected for cross-protection must be effective over a long period. Based on less impact in the 4th and 5th year (Fig. 2), ArMV-Ta may be very useful for cross-protection because grape is a perennial crop.

Our data showed no significant differences between field performance of 2 different infected Chardonnay clones (Tab. 1). This observation is of particular interest for the use of cross protection as a control strategy because many vineyards are plant-

ed with combinations of clones. It is likely that a mild strain would be effective on several clones of a *V. vinifera*.

The time consuming field experiments described here with infected grapevines would not be necessary if viral strains could be screened in the greenhouse on systemic herbaceous hosts. However, we found that although GFLV-CB844 caused mild symptoms on *C. quinoa* and GFLV-F13 severe symptoms, they both caused similar reactions on grapevines. Identical observations were obtained with other ArMV or GFLV strains not described here.

Our results also showed that vein mosaic had no impact on the field performance of either healthy or fanleaf infected vines (Tabs. 2 and 3). Vein mosaic which etiology is unknown has been reported as a minor virus-like disease (LEGIN and VUITTENEZ 1973; BOVEY *et al.* 1980; PEARSON and GOHEEN 1988). The horticultural data presented in this communication confirmed these previous observations.

Fanleaf degeneration is a very damaging viral disease which is impossible to control in established vineyards. We showed previously that ArMV inoculated *C. quinoa* prevented the deleterious effects of severe GFLV strains (HUSS *et al.* 1989). It would be of particular interest to test whether ArMV-Ta infected grapevines are protected after challenge inoculation with severe GFLV strains. Even if ArMV-Ta has small potential to reduce vigor and production, this mild strain might render grapevines less susceptible or even tolerant to more severe strains. Challenge inoculated grapevines can be obtained by graft inoculation or by natural transmission via nematodes either under controlled conditions in the greenhouse or in naturally contaminated vineyards. The ability to cross-protect grapevines against severe strains is now being investigated under field conditions.

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