Vitis 22, 40—50 (1983)

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Grapevine yellow speckle agent implicated in the aetiology of vein banding disease

by

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Die Bedeutung des Erregers von Grapevine yellow speckle in der Aetiologie von Vein banding disease

Zusammenfassung. — Aus einem Sultana-Klon, der an Vein banding und Yellow speckle erkrankt war, wurde der Vein-banding-Stamm des Fanleafvirus in gereinigter Form gewonnen; dieser Stamm besaß nicht mehr die Fähigkeit, an den Blättern gesunder Reben der Sorten Mission Seedling 1, LN 33, Cabernet Franc und Mataro Vein-banding-Symptome auszulösen. Dasselbe Virusisolat erzeugte jedoch in Verbindung mit Yellow speckle disease die gleichen Blattsymptome, wie sie durch die ursprüngliche Virusquelle von Vein banding disease bei LN 33, Cabernet Franc und Mataro induziert wurden. Die bei Yellow speckle beobachteten Blattsymptome waren vom Typ her bei allen Rebsorten identisch mit den Symptomen der Mischinfektionen von Yellow speckle und Vein-banding-Virus; allerdings konnte der Grad der Symptomausprägung bei Sultana, LN 33 und Cabernet Franc jahrgangsweise variieren.

Es wird die Hypothese aufgestellt, daß die mit Vein banding verbundenen Blattsymptome auf eine Yellow-speckle-Infektion zurückgehen und durch eine Mischinfektion mit Fanleafvirus verstärkt werden.

Introduction

Grapevine yellow speckle disease was reported by TAYLOR and WOODHAM (1972) to be caused by an unknown graft-transmissible agent that induced leaf symptoms sometimes indistinguishable from those of vein banding disease (GOHEEN and HEWITT 1962). This latter disease is widely considered to be caused by the vein banding strain of fanleaf virus (HEWITT *et al.* 1970, VUITTENEZ 1970, HEWITT and BOVEY 1979). Unlike the strains of fanleaf virus, the yellow speckle agent was not sap-transmitted to herbaceous plants nor was it eliminated from plants derived from shoot-tips propagated from vines grown at 38 °C for up to 11 months. Furthermore, TAYLOR and WOODHAM reported that clones of grape cultivars, which were symptomless in California, expressed yellow speckle disease when grown in Victoria. The presence of yellow speckle disease in Californian vines was later confirmed by MINK and PARSONS (1975) who indexed clones in controlled-environment chambers.

The viruses from different vein banding disease sources have been purified from herbaceous hosts and successfully returned to grapevines by MARTELLI and HEWITT (1963) and TAYLOR and HEWITT (1964), but the experimentally infected vines in both studies failed to reproduce the diagnostic vein banding symptoms even though some plants developed symptoms typical of fanleaf disease. This lack of conclusive evidence needed to fulfil Koch's postulates, together with the similar type of leaf symptom associated with both yellow speckle and vein banding diseases, stimulated us to further investigate vein banding disease.

Our previous indexing of all available vein banding disease sources and of fanleaf virus-free propagules derived through heat treatment has revealed that all our sources are co-infected with yellow speckle disease. This paper compares the leaf symptoms

induced by an isolate of the vein banding strain of fanleaf virus with that of yellow speckle disease, both singly and combined in different grapevine cultivars, and presents evidence that the yellow speckle agent is involved in the aetiology of vein banding disease.

Materials and methods

Verification of the vein banding disease

We used a clone of *Vitis vinifera* L. cv. Sultana (syn. Thompson Seedless) as the original source because it habitually expressed leaf symptoms identical to the vein banding disease described by GOHEEN and HEWITT (1962). This suspected disease, together with the associated strain of fanleaf virus was verified by the following widely recognised criteria (HEWITT *et al.* 1970):

(a) the reproduction of similar symptoms in graft-inoculated *V. vinifera* L. cultivars (Cabernet Franc, Cabernet Sauvignon, Mataro and a seedling of Mission, named Mission Seedling 1);

(b) the induced expression of ringspot, line-pattern and urticado reactions in graft-inoculated *V. rupestris* SCHEELE cv. St. George plants;

(c) the induced vein-clearing symptoms in *Chenopodium quinoa* WILLD. following mechanical inoculation with nicotine extracts of young Sultana leaves;

(d) the virus (p.FVB) partially purified by the method of HARRISON and NIXON (1960) from infected *C. quinoa* plants was shown to be an isometric particle approx. 30 nm in diameter, and to be serologically identical to the Californian vein banding antiserum.

Return of the virus (p.FVB) to grapevines

Healthy young Mission Seedling 1 plants were treated as described by HEWITT and CORY (1964) to produce etiolated growth suitable for inoculation. The laminae dusted with carborundum, were rubbed with the partially purified virus (p.FVB) obtained from infected *C. quinoa* plants. The inoculated vines were grown in containers under glasshouse and shadehouse conditions. During the following spring, we ascertained infections by leaf assays on *C. quinoa*. Positively infected vines were kept as a source of p.FVB for further comparative studies.

In addition, chip-buds from infected vines were used to inoculate 3 young plants of St. George and of LN 33 which were maintained under shadehouse conditions for 2 seasons, then planted in the field. These plants were regularly inspected for disease symptoms over 8 years.

Sources of yellow speckle disease (YS)

The YS isolates studied in the following experiments were from two *V. vinifera* sources:

(1) a fanleaf virus-free clone of Mission (Foundation Vineyard F9.V9) imported from the University of California, Davis, in 1963. Young plants of this clone in the field have always expressed leaf symptoms which, when severe, were indistinguishable from those of vein banding disease.

(2) a heat-treated Sultana clone derived from a shoot-tip propagated from the source vine of vein banding disease following 87 d at 38 °C (GOHEEN *et al.* 1965). Subsequent indexing of this clone proved that the vein banding-associated fanleaf virus had been eliminated and that the yellow speckle infection remained. Chip-buds from

this clone were used to graft-inoculate virus-free Cabernet Franc, Mataro and LN 33 vines to create further YS sources.

Comparison of vein banding and yellow speckle diseases

Test 1. — In spring 1975, 3 young Sultana and 2 young Mission vines with YS infections were graft-inoculated with chipbuds from Mission Seedling 1 plants infected with p.FVB by previous mechanical inoculation. These and corresponding non-inoculated plants were grown in containers under glasshouse and shadehouse conditions until spring 1976, when all were planted in the field. The replicates within each treatment were 2 m apart with a 3 m space between treatments. During the following spring, a sample of young leaves from each inoculated vine was assayed on *C. quinoa* to confirm virus infection.

Test 2. — In spring 1975 and 1976, young vines from both healthy (H) and YS-infected sources of Cabernet Franc, Mataro and LN 33 were inoculated with chipbuds from either of two infected sources:

(i) the original vein banding-diseased Sultana clone which was infected also with speckle disease (FVB + YS) or (ii) the purified vein banding-associated virus maintained in Mission Seedling 1 plants (p.FVB). These combinations plus the corresponding non-inoculated vines created the following six treatments in each cultivar: H; H + (FVB + YS); H + p.FVB; YS; YS + (FVB + YS); YS + p.FVB. Three replicates of each treatment were created in 1975 and two in 1976. All vines were grown in containers for the 1st growing season and during the following spring were planted in the field. Each cultivar was in a separate row spaced 3 m apart and the six treatments were randomised within each of five blocks with 2 m between vines. Three blocks were planted in spring 1976 and two in 1977. Each winter, the vines were spur-pruned at ground level and the new seasons' growth was trained on a vertical stake. Young leaves from those vines inoculated with the original or purified virus were assayed on *C. quinoa* during the 2nd to 4th spring after inoculation to confirm infection.

Assessment of symptoms

The vines in each test were regularly inspected and assessed visually during 4 or more years. In late autumn of the 2nd to 4th year following inoculation, prior to senescence and any loss of leaves, all leaves were stripped from the vines, graded according to

Table 1

Mean percent healthy leaves associated with yellow speckle disease (YS) and the combined infection of yellow speckle with the vein banding-associated fanleaf virus (p.FVB) in Sultana and Mission

Durchschnittlicher Prozentsatz gesunder Blätter der Sorten Sultana und Mission bei Yellow speckle disease (YS) und bei Mischinfektion von Yellow speckle und dem Vein-banding-Stamm des Fanleafvirus (p.FVB)

Cultivar	Year after inoculation	Treatment		L.S.D. (P=0.05)
		YS	YS + p.FVB	
Sultana	3	97.7	86.5	11.1
	4	94.6	87.2	
Mission	3	81.0	63.0	17.0
	4	67.1	66.1	

the nature and severity of symptoms, and counted. Data obtained from the four treatments incorporating YS were statistically analysed for differences in (a) percent healthy leaves per vine and (b) the weighted mean rating per affected leaf. The severity of symptom on each affected leaf was rated by the following scale where 1 = very mild; 3 = mild; 9 =moderate; 18 = severe. These ratings were devised from selected leaf standards within each cultivar and represented the relative affected portion of individual leaves.

Results

The successful return of the fanleaf virus isolate (p.FVB) to Mission Seedling 1 receptors was proven by indexing tests on *C. quinoa.* None of these plants expressed symptoms within the 10 years that followed inoculation. Furthermore, chip-buds from these vines induced in St. George vines the typical leaf symptoms associated with fanleaf virus infection. However, similarly graft-inoculated LN 33 vines did not express any symptoms during the 8 years after inoculation but the presence of the virus was confirmed by leaf assays on *C. quinoa.*

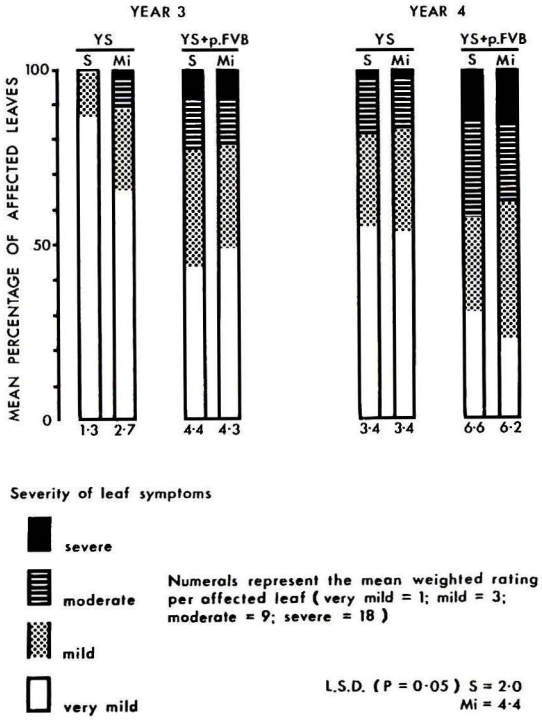


Fig. 1: The severity of leaf symptoms induced in Sultana (S) and Mission (Mi) by yellow speckle disease (YS) and the combined infection of yellow speckle with the purified strain of fanleaf virus associated with vein banding (YS + FVB).

Die Ausprägung der Blattsymptome bei Sultana (S) und Mission (Mi) bei Yellow speckle disease (YS) und Mischinfektion von Yellow speckle mit dem gereinigten Vein-banding-Stamm des Fanleafvirus (YS + FVB).

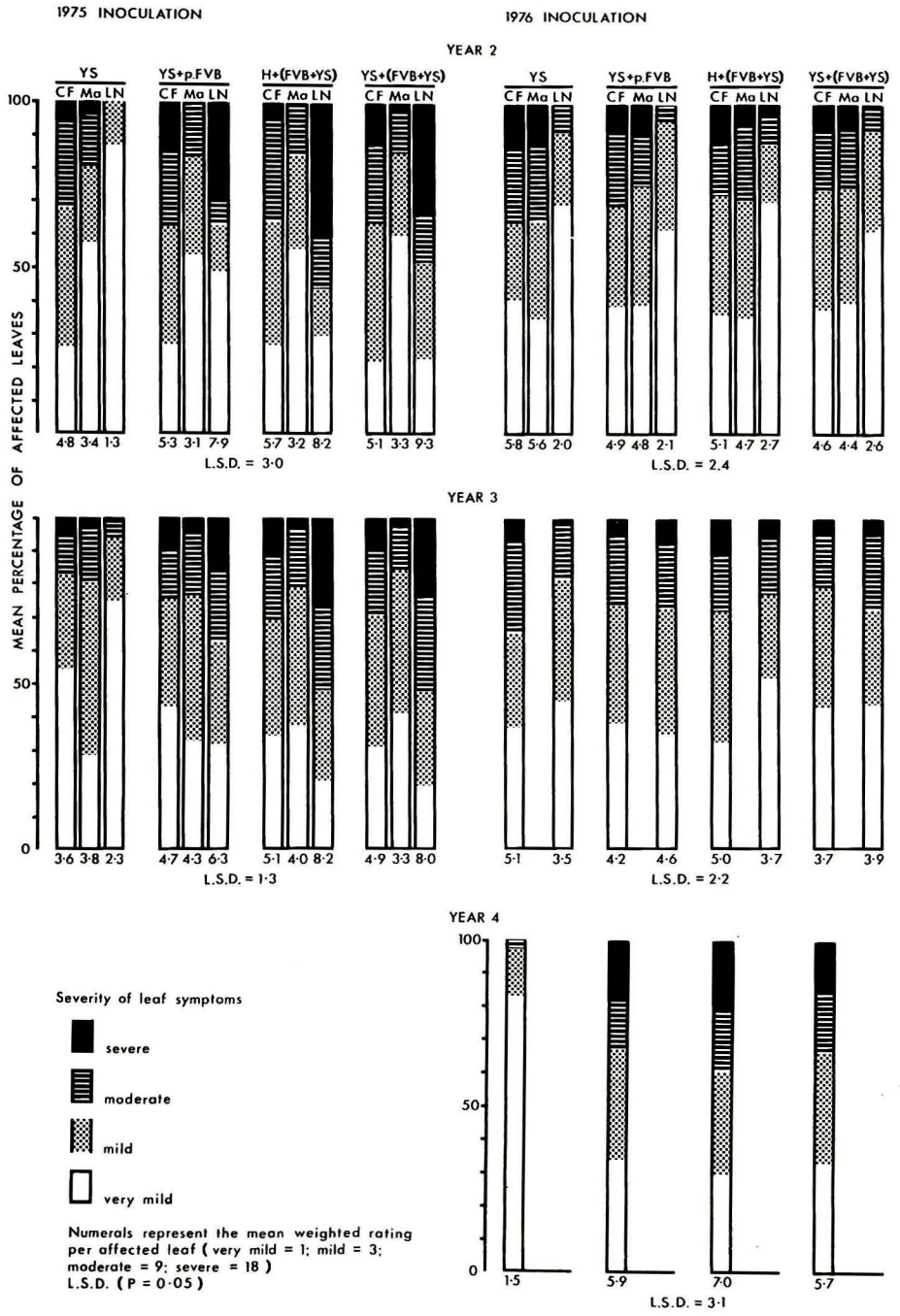


Fig. 2: The severity of leaf symptoms induced in healthy (H) Cabernet Franc (CF), Mataro (Ma) and LN 33 (LN) inoculated with the following, either singly or combined: vein banding-diseased Sultana clone (FVB + YS); yellow speckle disease (YS), derived from a heattreated sub-clone of the vein

Table 2

Mean percent healthy leaves associated with healthy (H) Cabernet Franc, Mataro and LN 33 inoculated with the following, either singly or combined: vein banding-diseased Sultana clone (FVB+YS); yellow speckle disease (YS), derived from a heat-treated sub-clone of the vein banding diseased Sultana clone; the purified vein banding-associated fanleaf virus (p.FVB), isolated from the vein banding-diseased Sultana clone

Durchschnittlicher Prozentsatz gesunder Blätter der Sorten Cabernet Franc, Mataro und LN 33 bei einfacher oder Mischinfektion · H: gesund; FVB+YS: an Vein banding erkrankter Sultana-Klon; YS: Yellow speckle disease von einem wärmebehandelten Subklon des an Vein banding erkrankten Sultana-Klons; p.FVB: gereinigter Vein-banding-Stamm des Fanleafvirus aus dem an Vein banding erkrankten Sultana-Klon

Inoculation	Cultivar	Year[1])	Treatment						L.S.D.[2]) (P= 0.05)
			H	H+ p.FVB	YS	H+ (FVB +YS)	YS+ p.FVB	YS+ (FVB +YS)	
1975	Grouped [3])	2	100	100	94.3	91.1	90.4	87.4	2.8
	Grouped	3	100	100	92.1	83.2	87.6	87.4	3.4
1976	Grouped	2	100	100	89.1	87.5	86.9	84.2	4.3
	Grouped	3	100	100	88.2	83.1	84.8	86.9	3.7
	Cab. Franc	4	100	100	97.4	86.1	90.2	89.7	12.3

[1]) Year following inoculation.
[2]) L.S.D.'s apply to the four treatments which expressed symptoms.
[3]) Mean values of all 3 cultivars.

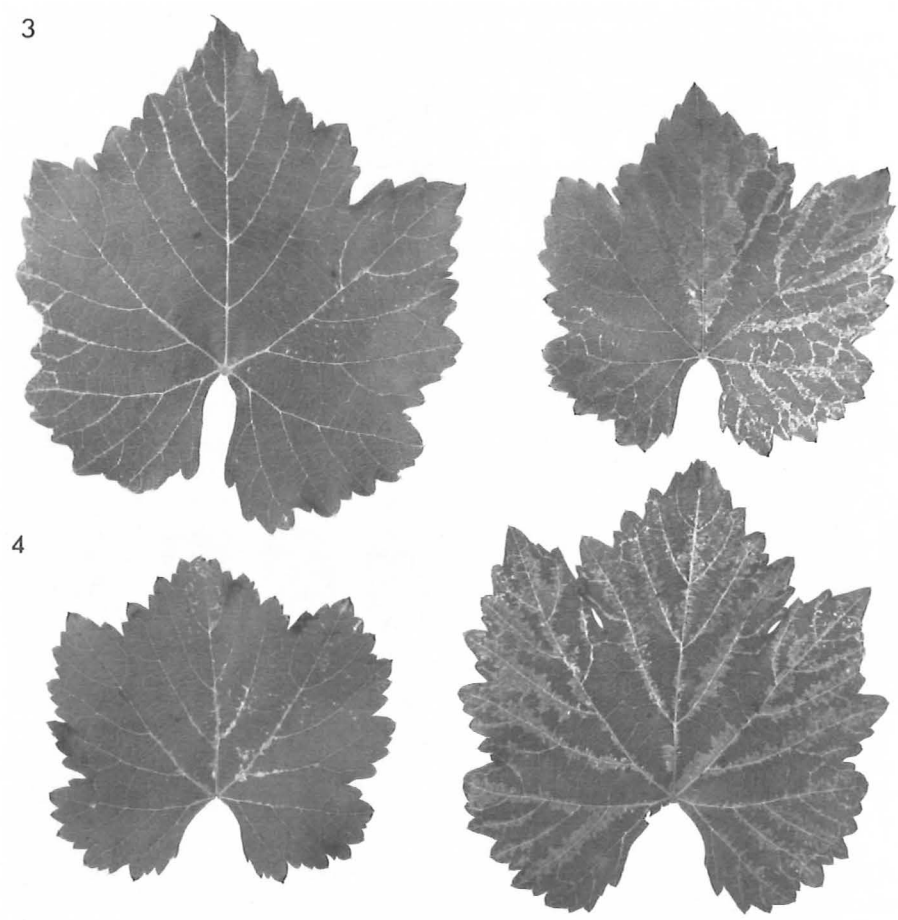
Analysis of vein banding and yellow speckle diseases

Test 1. — The analyses of leaf symptoms expressed by the YS-infected clones of Sultana and Mission and the same clones inoculated with p.FVB are shown in Table 1 and Fig. 1. Data from the 1st and 2nd year after inoculation were omitted because very few leaves in either treatment expressed symptoms. In all years, both YS and YS + p.FVB treatments expressed the same type of leaf symptom but produced some differences in the number of affected leaves and in the severity of symptoms. The addition of p.FVB induced more leaves with symptoms in both cultivars (less percent healthy leaves — Table 1) in year 3. Differences in year 4 were not significant, because in that year the YS-diseased vines had more leaves with symptoms in the mild to severe classes than in year 3.

As shown in Fig. 1, p.FVB increased the mean severity per affected leaf in Sultana in both year 3 and 4; the differences in Mission were not significant, probably due to insufficient replication. In both cultivars, the YS-infected vines had more leaves in the very mild class and fewer leaves in the severe symptom class than vines infected with YS + p.FVB. Typical examples of mild and severe leaf symptoms in Sultana and Mission are shown by Figs. 3 and 4.

banding-diseased Sultana clone; the purified vein banding-associated fanleaf virus (p.FVB), isolated from the vein banding-diseased Sultana clone.

Die Ausprägung der Blattsymptome bei Cabernet Franc (CF), Mataro (Ma) und LN 33 (LN) nach einfacher oder Mischinfektion. H: gesund; FVB + YS: an Vein banding erkrankter Sultana-Klon; YS: Yellow speckle disease von einem wärmebehandelten Subklon des an Vein banding erkrankten Sultana-Klons; p.FVB: gereinigter Vein-banding-Stamm des Fanleafvirus aus dem an Vein banding erkrankten Sultana-Klon.

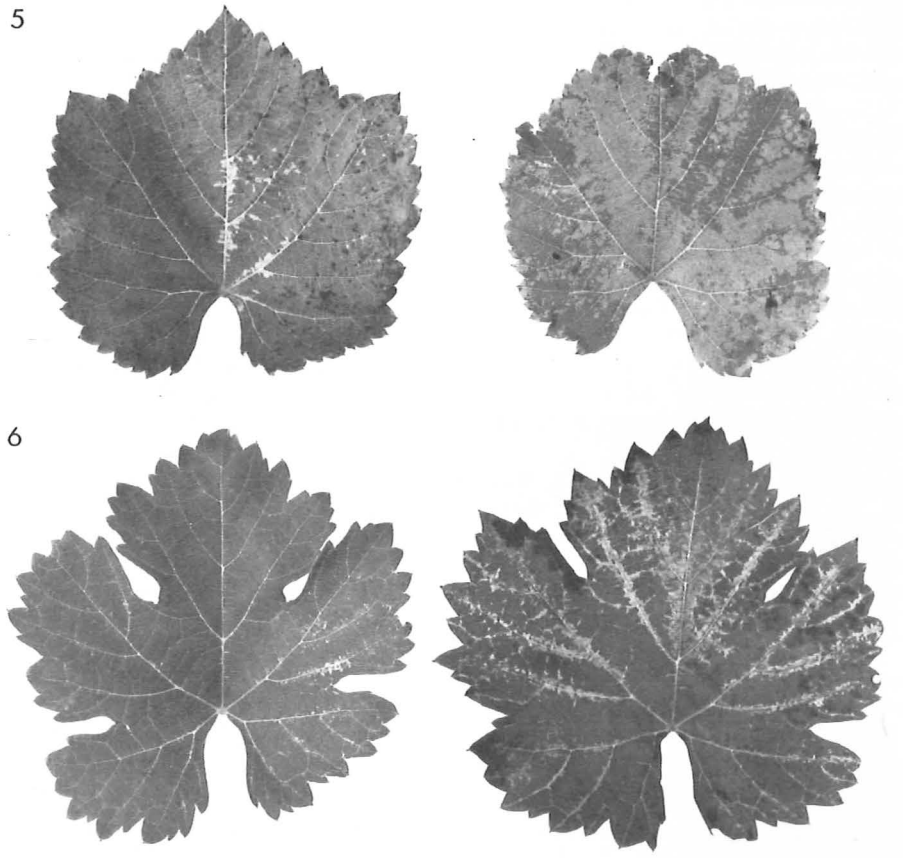


Figs. 3—6: Rating of leaf symptoms induced in Sultana (Fig. 3); Mission (Fig. 4); LN 33 (Fig. 5) and Cabernet Franc (Fig. 6), used to evaluate the treatments shown in Figs. 1 and 2. Mild symptoms (left), severe (right).

Klassifizierung der bei Sultana (Abb. 3), Mission (Abb. 4), LN 33 (Abb. 5) und Cabernet Franc (Abb. 6) ausgelösten Blattsymptome, die den Befunden der Abb. 1 und 2 zugrunde liegen. Links schwache, rechts starke Symptome.

Test 2. — Each vine inoculated with the original vein banding-diseased source or with the p.FVB component was verified as virus-infected by leaf assays in *C. quinoa.* The analyses of leaf symptoms induced in Cabernet Franc, Mataro and LN 33 vines in the 2nd to 4th year after inoculation are given in Table 2 and Fig. 2. In each year, all treatments within each cultivar had similar numbers of leaves. The mean percent value for healthy leaves of all 3 cultivars are presented (Table 2) because the cultivar × treatment interaction was not significant and there were no detectable trends.

No healthy vine or healthy vine inoculated with p.FVB expressed leaf symptoms during 4 years of observation within the test period or in 2 subsequent years. The leaf symptoms induced by the other four treatments in each cultivar were similar in nature and differed only in the severity of expression.



The three combinations of yellow speckle disease with the vein banding-associated virus created in 1975, namely H + (FVB + YS), YS + (FVB + YS) and YS + p.FVB, induced more leaves with symptoms than the corresponding YS treatment alone in year 2 ($P < 1\ \%$) and year 3 ($P < 0.1\ \%$). However, considerable variability in the weighted rating of symptoms occurred between cultivars and the cultivar × virus interaction was significant in both years, $P < 1\ \%$ and $P < 0.1\ \%$, respectively. Only LN 33 reliably showed the increased severity of leaf symptoms associated with the YS plus FVB combinations (Fig. 2). The YS-infected LN 33 vines had more leaves in the very mild class and fewer leaves in the severe class of symptoms. During year 4 and following years, the vines were visually assessed in the field because the leaves were too numerous for detailed analysis. In year 4, the YS plus FVB combinations again induced more severe leaf symptoms than YS alone in LN 33 but not in Cabernet Franc or Mataro. In year 5, the increased severity of symptoms associated with YS plus FVB combinations were expressed in all three cultivars; very obviously in LN 33 and Cabernet Franc and to a lesser degree in Mataro.

The duplicated treatments created in 1976 failed to show consistent differences in the number of leaves affected or in the weighted rating of symptoms between YS and the YS plus FVB combinations in any cultivar during the 3 years following inoculation. However, in the 4th year after inoculation, obvious differences in symptom severity

were detected in the field in LN 33 and Cabernet Franc. In Cabernet Franc, the symptom severity per affected leaf for the YS treatment was much less than that for the same vines in previous years (Fig. 2). This reduced severity was mainly due to fewer leaves in the moderate symptom class and no leaves with severe symptoms.

Examples of mild and severe ratings of symptoms in LN 33 and Cabernet Franc are illustrated by Figs. 5 and 6. The mottle-type symptom induced in LN 33 and the vein banding-type symptom in Cabernet Franc, Mataro, Sultana and Mission, although associated with YS treatments, were more frequent when YS was supplemented with FVB.

Although none of the H + p.FVB treatments developed leaf symptoms, the YS + p.FVB treatments expressed symptoms similar to those induced by the original vein banding-diseased Sultana clone (FVB + YS) in each of the Cabernet Franc, Mataro and LN 33 cultivars.

Discussion

In this study of the aetiology of vein banding disease, we have demonstrated the following:

(1) The vein banding strain of fanleaf virus purified from a Sultana clone infected with both vein banding and yellow speckle diseases, did not induce any leaf symptoms in healthy Mission Seedling 1, LN 33, Cabernet Franc or Mataro grapevines. These inoculated vines have remained symptomless for periods up to 10 years after inoculation.

(2) The combination of the purified fanleaf virus and yellow speckle disease reproduced a range of leaf symptoms similar to those induced by the original vein banding-diseased source in LN 33, Cabernet Franc and Mataro cultivars.

(3) The presence of the virus associated with vein banding disease considerably increased the severity of yellow speckle symptoms in cultivars less sensitive to YS (viz. LN 33 and Sultana), and in seasons less favourable for expression of yellow speckle symptoms.

(4) The severity of yellow speckle disease varied extremely, both within a cultivar between seasons and between cultivars within a season. The symptoms when severe in Sultana, Mission, Cabernet Franc and Mataro, could not be differentiated from those of vein banding disease described by GOHEEN and HEWITT (1962). Our findings, obtained with two sources of YS in comparative environments and years, have confirmed the observations of TAYLOR and WOODHAM (1972) who reported the extreme variability of symptom expression to be related to cultivar, age of plants, environmental and seasonal factors. More recently, SHANMUGANATHAN and FLETCHER (1980) have reported that different isolates of YS caused differences in the intensity and pattern of leaf symptoms.

GOHEEN and HEWITT (1962) recommended Mission and Thompson Seedless as good indicators for vein banding disease. We have failed to find YS-free clones of these 2 cultivars in our testing of 70 clones of Sultana in Australia or in 2 clones of Thompson Seedless and 2 clones of Mission imported from California. Our findings suggest the possibility that these cultivars in California carry an inapparent yellow speckle infection.

The currently accepted aetiology of vein banding disease seems based on the association of fanleaf virus with all known vein banding-diseased sources. Both MARTELLI and HEWITT (1963) and TAYLOR and HEWITT (1964) reasoned that the failure of their respective purified vein banding virus isolates to induce typical symptoms of vein banding disease in the inoculated vines was due to insufficient time for expression. MAR-

TELLI and HEWITT considered that the vein banding symptoms would be expressed in the following season but mentioned that the continued absence of vein banding symptoms could indicate "a composite disease". TAYLOR and HEWITT concluded that the fanleaf-type symptom was dominant and less sensitive to cultivar and environment than the vein banding symptom.

We recognise that our work is limited by the investigation of only one vein banding-diseased source. However, our indexing work over 16 years has failed to detect a vein banding-diseased source free of yellow speckle infection. Also the other recognised strains of fanleaf virus were not available for testing. We believe that our results do not support the established view that vein banding disease is caused by the vein banding strain of fanleaf virus. Our findings clearly show that yellow speckle is an essential component in the expression of vein banding disease. We present the hypothesis that vein banding symptoms are due to yellow speckle infection, intensified by co-infection with fanleaf virus.

The variable and sometimes severe expression of yellow speckle disease would make it extremely unwise to diagnose fanleaf virus infection solely on the basis of vein banding symptoms in anyone year in the field.

In a recent description of virus-like diseases in grapevines by BOVEY *et al.* (1980), it was suggested that yellow speckle leaf symptoms could be confused with the diagnostic symptoms of yellow mosaic, vein banding, yellow vein and chrome mosaic diseases. Following our experience with vein banding disease, we further speculate that yellow speckle disease could be involved in the aetiology of other diseases where the diagnostic leaf symptoms are based on chrome-yellow variegations.

Summary

The vein banding strain of fanleaf virus, purified from a vein banding and yellow speckle diseased Sultana clone, lost the ability to induce vein banding leaf symptoms in healthy Mission Seedling 1, LN 33, Cabernet Franc and Mataro grapevines. The same virus isolate in combination with yellow speckle disease produced the same range of leaf symptoms as those induced by the vein banding disease source in LN 33, Cabernet Franc and Mataro vines. The leaf symptoms associated with yellow speckle and yellow speckle combined with the vein banding virus within each cultivar were identical in type although differences in severity occurred in Sultana, LN 33 and Cabernet Franc in some years.

We present the hypothesis that the leaf symptoms associated with vein banding disease are due to a yellow speckle infection, intensified by co-infection with fanleaf virus.

Acknowledgements

We acknowledge the late Dr. H. F. DIAS, Vineland Station, Ontario, Canada, for the gift of vein banding antiserum. We also thank Mr. K. M. CELLIER, CSIRO Division of Mathematics and Statistics for all statistical analyses; and Mr. E. A. LAWTON, CSIRO Division of Horticultural Research for the photographs.

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Eingegangen am 13. 7. 1982

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