At left, yellow mosaic symptoms in Cabernet Sauvignon. Above, vein banding and fruit symptoms common in Cabernet Sauvignon grapes infected with grapevine fanleaf virus. Poor set (seen above) can result in dramatic reductions in yield.

Two-year study in San Joaquin County indicates . . .

Sampling procedures to find nepoviruses in grapevines need improvement

Deborah A. Golino ❑ Paul Verdegaal ❑ Adib Rowhani
M. Andrew Walker

Two debilitating grapevine diseases, fanleaf and yellow vein, are caused by nepoviruses. Once these viruses are established in vineyards along with their nematode vectors, they are extremely difficult to eradicate. Since the use of infected propagating wood can spread the diseases further, the development of rapid diagnostic procedures for these viruses is highly desirable. However, a 2-year study on the identity and incidence of nepovirus-infected grapevines in San Joaquin County vineyards indicates that sampling procedures and ELISA protocols will have to be improved before this virus assay can be used reliably in nursery certification programs.

The grapevine nepoviruses, nematode-transmitted polyhedral viruses, seriously reduce vineyard productivity and longevity. Grapevine fanleaf virus (GFLV), transmitted by the nematode, *Xiphinema index*, is known to be widespread in California. Grapevine yellow vein, caused by tomato ringspot virus (TomRSV), can be transmitted by *X. americanum*, *X. californicum* or *X. rivesi*.

Although TomRSV is common in vineyards in eastern states, it has not been reported often in California vineyards. However, the same virus is widespread in other fruit crops in California, including peach, apple and cherry. It is possible that the disease caused by TomRSV has been confused with that caused by GFLV. The symptoms caused by the two viruses are difficult to distinguish in the field because both cause weak growth, poor fruit set and vine decline. Common leaf symptoms in grape produced by both fanleaf and yellow vein include oak leaf patterns, yellow mosaic and vein banding.

Commercial grape production was established in San Joaquin County around 1850. Warm days and cool nights influenced by the Delta region to the west encouraged early use of many varieties for both wine and table grape production. The area includes some of the oldest vineyards in California, dating back to the turn of the century. Most of the older vineyards in the county are own-rooted Tokay and Zinfandel.

Introduction of St. George rootstock, among others, increased the spread of viruses as neighbors traditionally shared propagation material, and many early St. George rootstock selections in California were virus infected. Because much of the acreage was planted before virus-tested, certified grape scions and rootstocks were available to growers, the probability of virus infection countywide was increased. Frequent observations have been made by the authors and others in many San Joaquin vineyards of the leaf symptoms commonly associated with nepovirus infection. However, other vineyard problems can cause similar symptoms. Until recently, it has been difficult to determine whether a particular symptom is due to infection by GFLV or TomRSV.

We were interested in finding out how common GFLV and TomRSV are in San Joaquin County vineyards. The serological test known as ELISA (enzyme-linked immunosorbant assay) has made possible simple, inexpensive testing that distinguishes between healthy vines, vines infected with GFLV and vines infected with

TomRSV. The ELISA test uses highly specific antibody molecules to detect the presence of individual viruses. By using antibodies to each virus, the cause of disease can be identified. Past reports indicate that yellow vein is limited in distribution in California. We wanted to see whether this was still correct or whether this disease has become more widespread. Finally, long-term studies on the use of ELISA sampling for field detection of viruses are underway, and we hope to identify areas requiring additional study.

Potential sources of error in the use of the ELISA test for field virus detection include variation in test results due to uneven distribution of virus in different tissues, changes in virus titers due to tissue age, seasonal variation in viral titer, the existence of virus strains with different serological reactions and the possibility of false negative or positive reactions brought about by either operator error or faulty reagents. Before ELISA can be used routinely for quarantine and certification of grapes, more information is needed about the reliability of its use under field conditions. This study provided initial information on the use of ELISA for large-scale field testing.

Survey methods

An effort was made to sample vineyard diversity, including vineyards of different ages and varieties. Vineyards in which nepovirus symptoms had been observed, as well as those without any signs of disease symptoms, were sampled. When possible, vineyard history, including vineyard age, previous crops and presence or absence of viruslike symptoms, was recorded.

GFLV and TomRSV are most easily detected by ELISA early in the growing season, so sampling was conducted between May and July of 1990 and 1991. Each vineyard was sampled both years with the exception of three vineyards, which were removed in the winter of 1991.

In each of the 44 vineyards tested, a standard set of samples from 27 vines was collected. Samples were taken beginning in a row designated as row 1 for sampling purposes. A pooled sample of about 0.2 g of young succulent tissue was taken from vines 1, 2 and 3 in that row.

Past work has shown that GFLV and TomRSV can be detected in pooled samples, even if only one in five vines is virus infected. Vines 21 to 23 constituted the second sample; vines 41 to 43 provided the third and final sample for row 1. The same vine-sampling pattern for three samples, each representing three vines, was used for rows 21 and 41. This resulted in a total of nine samples from a particular site, each of which included tissue from three vines. Thus, 27 vines were sampled from each vineyard. If a given vineyard did not contain enough rows or vines for this pattern, the size of the sampling grid was reduced proportionally so that the same number of samples could be collected. In total, 396 samples were taken from 44 vineyards for this study from 1,188 vines.

Yellow mosaic symptoms in Tokay grapevines caused by grapevine fanleaf virus.

The ELISA protocol that was used involved the production of what is known as a $F(ab')_2$ fragment, which is used to coat the polystyrene plate in which the test is conducted. This type of ELISA test had been previously shown to work effectively with both GFLV and TomRSV. Unlike some other ELISA procedures, the $F(ab')_2$ ELISA allows use of a single antibody in combination with commercial enzyme conjugates, resulting in consistent results with minimum background levels.

Tissue samples were collected in the field directly into labeled vials containing 2 ml of cold sample buffer. Samples were refrigerated overnight if they were to be processed the next day or frozen immediately for processing within 2 weeks. A sample volume was sufficient to test for GFLV and TomRSV simultaneously from a single sample.

A few modifications of standard procedures were used for the ELISA test. We have found an $F(ab')_2$ type ELISA test to be simple and effective since it allows us to purchase commercial reagents for a critical enzymatic step in the procedure. Also, because grape tissue is very acidic, it is important to use a high pH sample buffer to get reliable results. Carbonate buffer at pH 9.6 greatly improves the reliability of ELISA tests run on grapevines.

ELISA results were scored as positive if sample values were at least three times the value of healthy control tissue after adjustment for the plate background levels. Values which were two to three times the healthy value were considered suspect; in routine sampling, these vines would need to be retested. Values lower than two times the background level were recorded as negative for the tested virus. In our analysis, if 1 year's value was at cautionary levels, the reading of the alternate year was used as the value in the results and discussion.

Results and discussion

Of the 44 sites sampled, only 17 were free from nepovirus disease symptoms. In the 27 vineyards with symptomatic vines, symptom severity did vary considerably from vine to vine within a vineyard and from year to year. Although some grape varieties seemed to demonstrate a predominance of a particular symptom type (that is, Tokay vines frequently showed strong yellow mosaic symptoms), careful examination revealed all three symptom types were present in most vineyards in which any symptoms could be observed.

No positive ELISA readings for TomRSV were recorded for any of the vineyards sampled. Two suspect sample values were recorded in 1990 and six in 1991. However, no vine sample sets were in the cautionary range more than once. Positive controls, samples with known TomRSV infections, were all strongly positive, as would be expected. TomRSV is known to exist in a number of serologically distinct strains. Although it is possible that the cautionary values were produced by reactions with samples from vines infected with a serologically unique strain of TomRSV that reacted weakly with the antisera in the ELISA test, they are probably as likely to represent reactions with samples that produced high background reactions or other types of experimental variation.

Of the 396 samples processed for the study, 153 tested positive for GFLV in 1990. All but 80 samples gave identical results in the GFLV ELISA in 1990 and 1991. Of these 80, most represented value changes between the cautionary level and either negative or positive values. Those vine sample sets were analyzed as representing the nonborderline value, that is, if a sample was negative 1 year and cautionary the next, we considered the sample negative. However, 29 of the 396 samples (7.3%) were negative in the first year and positive in the second or visa versa. Those samples that went from negative to positive (20 of those 29) may represent new infections in vineyards where disease is spreading.

TABLE 1. Result of ELISA tests for tomato ringspot virus (TomRSV) and grapevine fanleaf virus (GFLV) in San Joaquin County vineyards in 1990 and 1991*

				No. samples infected§			
				TomRSV/total		GFLV/total	
Site	Cultivar†	Rootstock‡	Symptoms	1990	1991	1990	1991
1.	Chardonnay	OR	+	0/9	0/9	0/9	1/9
2.	Chardonnay/ Burger	Freedom	–	0/9	0/9	0/9	1/9
3.	Carignane	Dog Ridge	+	0/9	VR	5/9	VR
4.	Tokay	OR	+	0/9	0/9	1/9	9/9
5.	Burger	OR	+	0/9	VR	1/9	VR
6.	Tokay	OR	–	0/9	0/9	0/9	3/9
7.	Zinfandel	St. George	+	0/9	0/9	9/9	9/9
8.	Zinfandel	OR	+	0/9	0/9	6/9	5/9
9.	Zinfandel	St. George	+	0/9	0/9	9/9	9/9
10.	Burger	St. George	+	0/9	0/9	9/9	5/9
11.	Colombard	St. George	+	0/9	0/9	9/9	9/9
12.	Burger	NK	+	0/9	0/9	0/9	1/9
13.	Burger	St. George	+	0/9	0/9	5/9	5/9
14.	Tokay	OR	+	0/9	0/9	0/9	0/9
15.	Carignane	St. George	–	0/9	0/9	4/9	2/9
16.	Zinfandel	St. George	+	0/9	0/9	9/9	9/9
17.	Colombard	Harmony	+	0/9	0/9	1/9	2/9
18.	Zinfandel	St. George	+	0/9	0/9	3/9	5/9
19.	Burger/ Petite Sirah	St. George	–	0/9	0/9	2/9	3/9
20.	Zinfandel	St. George	+	0/9	0/9	3/9	6/9
21.	Burger	Freedom	+	0/9	0/9	7/9	5/9
22.	Tokay	OR	+	0/9	0/9	0/9	1/9
23.	Zinfandel	Grey Reisling	+	0/9	0/9	9/9	7/9
24.	Zinfandel	OR	+	0/9	0/9	6/9	7/9
25.	Zinfandel	Freedom	–	0/9	0/9	0/9	2/9
26.	Zinfandel	St. George	+	0/9	0/9	9/9	9/9
27.	Tokay	St. George	–	0/9	0/9	0/9	0/9
28.	Chardonnay/ Petite Sirah	St. George	–	0/9	0/9	0/9	1/9
29.	Tokay	OR	+	0/9	VR	3/9	VR
30.	Chardonnay/ Tokay	Freedom	–	0/9	0/9	3/9	5/9
31.	Chardonnay	Tokay	–	0/9	0/9	0/9	0/9
32.	Burger	St. George	+	0/9	0/9	9/9	9/9
33.	Chardonnay/ Tokay	Freedom	–	0/9	0/9	0/9	3/9
34.	Cabernet Sauvignon	039–16	-	0/9	0/9	0/9	0/9
35.	Zinfandel	Dog Ridge	–	0/9	0/9	7/9	7/9
36.	Tokay	OR	+	0/9	0/9	9/9	9/9
37.	Zinfandel	Dog Ridge	+	0/9	0/9	9/9	9/9
38.	Zinfandel	OR	–	0/9	0/9	0/9	0/9
39.	Colombard	OR	–	0/9	0/9	0/9	1/9
40.	Merlot/Tokay	Freedom	–	0/9	0/9	1/9	0/9
41.	Tokay	OR	+	0/9	0/9	1/9	2/9
42.	Tokay/ Colombard	St George	+	0/9	0/9	2/9	2/9
43.	Burger	St. George	–	0/9	0/9	1/9	0/9
44.	Cabernet Sauvignon/ Chenin Blanc	St. George	–	0/9	0/9	1/9	0/9

*A sample was considered infected if it produced an ELISA test value at least 3 times as high as healthy plant tissue.
†If 2 varieties, scion has been grafted over new scion/old scion.
‡OR = Own roots; NK = Not known.
§VR = Vineyard removed.

The other nine samples (about 2% of the total) that went from positive to negative are more difficult to explain. There are no known cases of grapevines recovering from either GFLV or TomRSV. They could represent changes in titer of the virus, inadequate controls or operator error.

Of the 44 vineyards, 27 contained vines with nepovirus type symptoms. Of these, while a single vineyard did not test positive for either TomRSV or GFLV infection, 25 vineyards contained GFLV-infected vines. Eight vineyards were nepovirus symptomatic and 100% infected for all vine groups sampled in both years.

Of the 17 vineyards without symptoms, 13 vineyards were GFLV ELISA positive. The other four tested as negative for infection. The absence of GFLV symptoms does not assure freedom from virus infection according to our observations.

Conclusion

A surprisingly high percentage of tested San Joaquin County vineyards were positive for GFLV. No infection with TomRSV was detected. It does not appear that the grapevine yellow vein strain of TomRSV accounts for much of the nepovirus disease observed in San Joaquin County.

The fanleaf, yellow mosaic and veinbanding symptoms so often observed in the county seem to be predominantly associated with the presence of GFLV. Owners of vineyards with GFLV would be well advised to sample for the fanleaf vector, *X. index*. If vineyards are not infested with the vector, replanting with certified, virus-tested nursery materials could substantially improve productivity.

Neither the presence nor absence of nepovirus-associated symptoms is sufficient to assess whether a vine is infected with GFLV. Sampling variation was observed during the 2 years of this study, and further study of sampling procedures will be necessary to achieve a higher level of confidence before ELISA can be routinely used in certification programs. Testing of critical propagation materials by ELISA, such as those in certified nursery blocks, will only be possible when reliable sampling procedures are established. This work is underway.

D. A. Golino is Research Plant Pathologist, USDA-ARS, Department of Plant Pathology, UC Davis; P. Verdegaal is Farm Advisor, San Joaquin County; A. Rowhani is Specialist, Department of Plant Pathology, UC Davis; and M. A. Walker is Assistant Professor, Department of Viticulture and Enology, UC Davis.

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