Field Serological Detection of Viral Antigens Associated with Grapevine Leafroll Disease

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ABSTRACT

Teliz, D., Tanne, E., Gonsalves, D., and Zee, F. 1987. Field serological detection of viral antigens associated with grapevine leafroll disease. Plant Disease 71:704-709.

A polyclonal antiserum produced against closteroviruslike particles purified from leafroll-diseased grapevines in New York was used to determine the best time and tissue type for field detection of the disease. Grapevine leafroll-associated viral antigens were detected by direct enzyme-linked immunosorbent assay in 8-yr-old Pinot noir vines in a commercial vineyard in New York during the 1986 growing season. The viral antigens were detected in phloem extracts of dormant cuttings after 6 mo of storage at 6 C. Viral antigens were first detected 15 days after budbreak in flowers at the stage of inflorescence swelling. Basal leaves were not reliable for field detection until bloom. Viral antigens were evenly distributed in shoots arising at the basal, middle, and apical portions of 1-yr-old canes. Besides flower clusters and leaves, the viral antigens were also detected in roots, fruit, fruit peduncles, tendrils, and bark tissue. In fruit, the viral antigens were detected in all stages, except when they were 2–4 mm in diameter. Virus was detected in symptomatic and symptomless leaves in diseased but never in healthy vines. Flower clusters and roots were the tissues from which the virus was first detected 15 days after budbreak in greenhouse-grown, diseased cuttings, whereas leaves did not became reliable virus sources until 28 days after budbreak. The rate of advance of the viral antigens in growing shoots appeared to vary according to the growth stage of the vines: 1) from budbreak to inflorescence swelling, the viral antigens were restricted to flower clusters and were not detected in leaves; 2) from inflorescence development to developing berries (25–75 days after budbreak), the viral antigens were detected in the leaves but did not reach the terminal ones; and 3) from berry touch stage until harvest, the viral antigens were detected in all leaves including the terminal leaf.

Grapevine leafroll (GLR) is one of the most important diseases of grapevine in the world. Diseased vines are characterized by downward rolling and a metallic yellow coloring of white cultivars or general reddening of leaf blades with green veins in red cultivars. Gradual reduction of yield and sugar content of fruits are results of the disease. Graft transmissibility and sensitivity to thermotherapy suggest a viral etiology, but the nature and cause of the disease have not been unequivocally determined. Researchers from several countries have related different types of viruslike particles to the disease: potyvirus (20), isometric particles (3), and closterovirus (3,9,10,13,15–17,22,23). Most of these works were based on electron microscope observations of tissues from diseased vines.

Gugerli et al (13) purified filamentous particles of closterovirus type and produced an antiserum with which enzyme-linked immunosorbent assay (ELISA) detection was successfully performed in a large number of different cultivars. Recently, Zee et al (26) purified closteroviruslike particles (NY-1) from leafroll-diseased Pinot noir vines in New York and developed a highly specific antiserum. A sensitive method like direct ELISA (4) and a specific antiserum are powerful tools to learn more about this important disease. The purposes of the present work were to determine the best time and tissue type for early detection of the disease and the distribution and movement of the viral antigens associated with GLR disease in grapevines during one growing season on the assumption that such data may have wider applicability in other GLR situations besides the one studied herein. We are equating the virus detected by NY-1 antiserum to GLR virus (GLRV) for the purposes of this paper, intentionally begging the question of whether NY-1 GLRV may, in fact, be representative of viruses causing GLR elsewhere.

Accepted for publication 5 March 1987 (submitted for electronic processing).

MATERIALS AND METHODS.

The field study was conducted in a commercial vineyard on the west side of Cayuga Lake in Seneca County, New York. Eight 8-yr-old Pinot noir vines infected with GLR were selected. Cuttings from four of the eight diseased vines were rooted and grown in a greenhouse for comparative observations.

Direct ELISA with NY-1 antiserum (26) (prepared against closteroviruslike particles from the same Pinot noir vines used in this study) was used to detect the viral antigens in leaf, flower, fruit, tendril, bark, and root tissues. Each leaf sample (40 mg) was collected with a no. 14 cork borer, always including a portion of the midvein or secondary veins. Flower samples consisted of an apical portion of one flower cluster per vine. Two slices of two or three berries and bark slices from internodal tissues were cut with a razor blade for the fruit and bark samples, respectively. The two most basal tendrils were sampled by cutting 2-cm sections of their terminal and middle portions. Two segments of 2 cm each of apical and more mature roots were sampled per vine. Leaf, flower, fruit, bark, root, and tendril samples were macerated in ELISA extraction buffer (4) at a dilution of 1:50. Direct ELISA was performed in Immulon 2 U-bottom plates (Dynatech Laboratories, Inc., Chantilly, VA), coated with 1 μg of γ-globulin per milliliter of coating buffer (4). The conjugate was used at a dilution of 1:2,000 in enzyme buffer, and the substrate, at a concentration of 1 mg of *p*-nitrophenyl phosphate per milliliter of substrate buffer (4). Basal leaf samples from healthy and GLR-diseased greenhouse-grown Pinot noir vines served as negative and positive checks, respectively. Optical density (A_{405nm}) was periodically measured in an MR 580 micro-ELISA Autoreader (Dynatech) whose optical density range was 0–1.5. The high sensitivity of NY-1 antiserum was expressed by development of color, so rapid that it frequently started before we finished filling the ELISA plate with the substrate. This is why the concentration of the γ-globulin had to be reduced from 2 to 1 μg/ml, the conjugate from 1:1,000 to 1:2,000, and the sampled tissues in extraction buffer from 1:20 to 1:50.

To determine the distribution of the viral antigens in grapevines during the 1986 growing season, assays on selected vines were initiated 15 days after budbreak (visible flower cluster stage, according to the phenological system of von Eichorn and Lorenz [25]). Samples of basal leaves and flower clusters from basal shoots were used. Subsequent samples were taken every 10–15 days

Table 1. Optical density readings (A_{405nm}) of samples from eight leafroll-diseased Pinot noir grapevines growing in the field and four vines growing in a greenhouse during 1986, measured by direct enzyme-linked immunosorbent assay using NY-1 antiserum prepared against closteroviruslike particles from these same diseased Pinot noir field vines

Phenological stage[a]	Sample	Vine 1	2	3	4	5	6	7	8	Mean	−CK[b]	+CK[b]
01: Winter dormancy	Bark	1.500	1.500	1.500	1.500	...	...	...	...	1.500	0.001[c]	1.222[c]
15: Inflorescence swelling	F[d] L[e]	0.045	0.016	0.012	0.024	0.006	0.020	0.007	0.047	0.022		
	F I[e]	0.198	0.285	0.275	0.372	0.205	0.062	0.079	0.136	0.202		
	G[d] L	0.000	0.056	0.001	0.000	...	...	...	...	0.014		
	G I	0.083	0.453	0.242	...	...	...	...	...	0.259		
	G R[e]	0.200	0.160	0.278	0.094	...	...	...	...	0.183	0.002	1.500
17: Inflorescence fully developed	F L B[f]	0.043	0.043	0.054	0.138	0.023	0.101	0.032	0.017	0.056		
	F L M[f]	0.072	0.054	0.053	0.048	0.033	0.012	0.037	0.026	0.042		
	F L A[f]	0.169	0.100	0.068	0.061	0.019	0.032	0.030	0.018	0.062		
	F I B	0.362	0.353	0.067	0.490	0.063	1.135	0.056	0.053	0.321		
	F I M	0.295	0.297	0.197	0.399	0.146	0.435	0.130	0.086	0.248		
	F I A	0.680	0.327	0.054	0.129	0.165	0.074	0.073	0.057	0.194		
	G L	0.004	0.090	0.076	0.067	...	...	...	...	0.059	0.002	1.500
19: Beginning of flowering	F L B	...	0.285	0.023	0.712	...	0.098	0.097	0.200	0.236		
	F L M	...	0.037	0.008	0.116	...	0.016	0.016	0.136	0.055		
	F L A	...	0.045	0.005	0.100	...	0.009	0.000	0.035	0.032		
	F R	0.128	0.113	0.145	0.173	0.096	0.295	0.132	0.185	0.159		
	G L	0.062	0.372	0.076	0.381	...	...	...	...	0.223	0.003	1.281

[a] Following the phenological system of von Eichhorn and Lorenz (25).
[b] Basal leaf samples from healthy (−CK) and leafroll-diseased (+CK) greenhouse-grown Pinot noir vines.
[c] Controls for bark assays included bark scrapings from leafroll-diseased (+CK) greenhouse-grown Pinot noir vines and from healthy greenhouse-grown Pinot noir vines and from dormant healthy Cabernet franc cuttings stored at 6 C for several months.
[d] Field-grown (F) or greenhouse-grown (G) vines.
[e] Samples from leaves (L), inflorescences (I), or roots (R).
[f] Samples from shoots arising at basal (B), middle (M), or apical (A) position of a cane.

throughout the growing season. Samples included leaves, fruit, fruit pedicels, roots, tendrils, and bark. To determine if the viral antigens were evenly distributed throughout the canes, leaf and flower samples from shoots arising at basal, middle, and apical positions in selected canes were assayed by ELISA. Distribution of the viral antigens in shoots was determined by periodic, systematic, and acropetal sampling of leaves. The relationship between ELISA results and symptoms was studied by careful observation of leaf symptoms at every sampling date. Internodal bark tissues were scraped from the inner bark of diseased dormant cuttings collected in April, stored at 6 C for 6 mo, and tested by direct ELISA against the NY-1 antiserum. Controls for bark assays included bark scrapings from dormant healthy Cabernet franc cuttings stored at 6 C for several months and from healthy and GLR-diseased greenhouse-grown Pinot noir vines.

Throughout our study, the maximum ELISA reading our negative controls recorded was 0.003; therefore, we did not set our positive threshold at the mean value plus twice or three times the standard deviation. Instead, we arbitrarily selected 0.050 readings as our positive threshold. All readings presented in the figures are an average of eight (field) or four (greenhouse) sampled vines (two ELISA wells per vine).

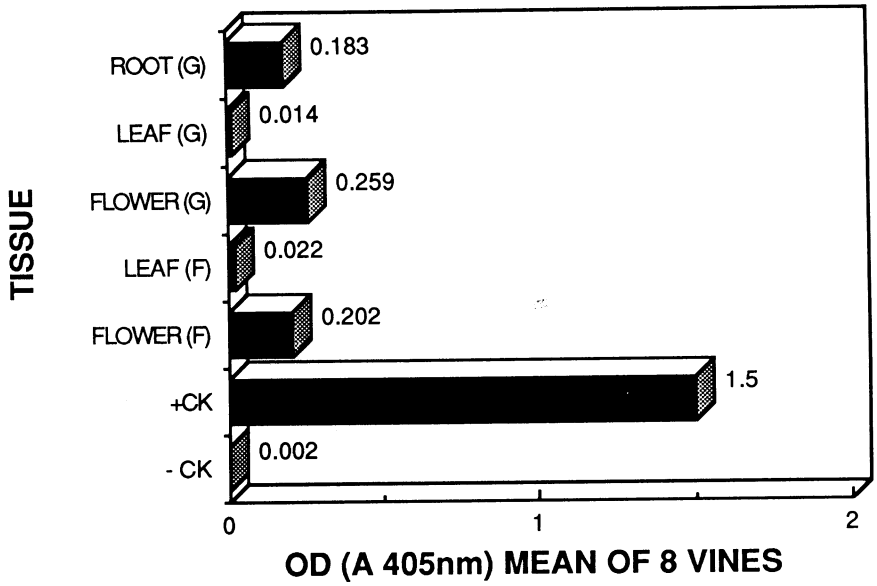

Fig. 1. Direct ELISA detection of viral antigens associated with grapevine leafroll (GLR) disease in flower clusters, leaves, and root samples of GLR-diseased Pinot noir vines in the inflorescence swelling stage (15 days after budbreak) growing in a commercial vineyard (F) or in the greenhouse (G). Basal leaf samples from healthy and GLR-diseased, greenhouse-grown Pinot noir vines served as negative and positive checks, respectively (minimum optical density for positive detection = 0.050).

RESULTS

The distribution of the viral antigens in the grapevines at different phenological stages, among the 40 stages listed by von Eichorn and Lorenz (25), was as follows:

Stage 01: winter dormancy. The viral antigens were detected in the bark tissue from dormant canes collected from diseased vines. The maximum ELISA reading (A_{405nm} = 1.5) was reached even earlier than with the positive checks (bark from GLR-diseased vines growing

in the greenhouse), which in the same time interval (12 min) reached an average reading of 1.222. The extract from bark of healthy Pinot noir greenhouse-grown vines and from healthy Cabernet franc cuttings stored at 6 C for several months (negative checks) gave an average reading of 0.001 (Table 1).

Stage 15: inflorescence swelling, flowers closely pressed together (15 days after budbreak in Seneca County in 1986). The viral antigens were detected in flower clusters in all eight vines in the commercial vineyard but not in any of the leaf samples. In the greenhouse-grown vines, the virus was also detected in flower clusters and roots of all four vines but in only one leaf sample (Table 1, Fig. 1).

Stage 17: inflorescence fully developed, flowers separating (25 days after budbreak). To determine the distribution of the viral antigens in the canes, samples of flower clusters and basal leaves were taken from shoots arising at basal, middle, and terminal positions in the canes of field-grown vines. Viral antigens were detected in flower clusters from all three positions (Fig. 2). Viral antigens were only detected in leaves of five of eight vines. Leaf samples in the greenhouse-grown plants yielded the virus in three of the four assayed vines (Table 1).

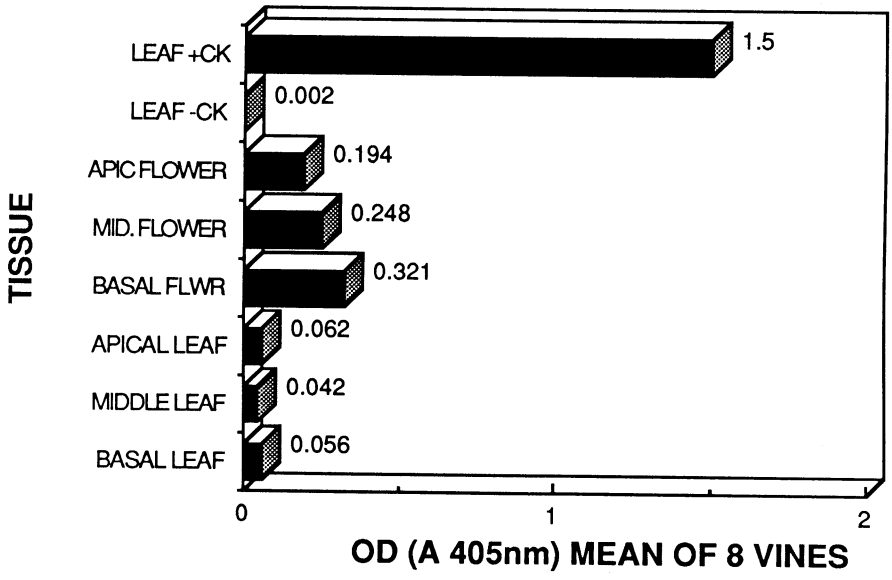

Fig. 2. Direct ELISA detection of viral antigens associated with grapevine leafroll (GLR) in flower clusters and leaf samples from GLR-diseased grapevines in a commercial vineyard at the inflorescence fully developed stage (25 days after budbreak). The antigens were detected in the flower samples of shoots arising from buds at basal, middle, and apical positions in the canes of the eight vines and in leaf samples of only five vines. Basal leaf samples from healthy and GLR-diseased, greenhouse-grown Pinot noir vines served as negative and positive checks, respectively (minimum optical density for positive detection = 0.050).

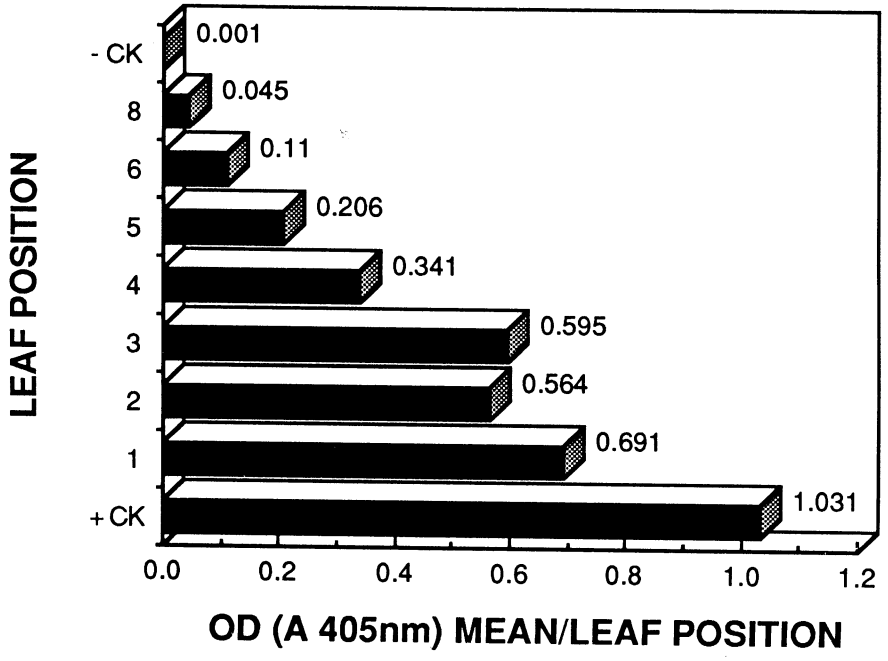

Fig. 3. Mean optical density (A_{405nm}) readings of samples from basal (1) to apical expanding leaves (8) from eight leafroll-diseased grapevines in a commercial vineyard at early flowering stage (38 days after budbreak). Basal leaf samples from healthy and leafroll-diseased, greenhouse-grown Pinot noir vines served as negative and positive checks, respectively (minimum optical density for positive detection = 0.050)

Stage 19: beginning of flowering (34 days after budbreak). The viral antigens were detected in leaf samples from basal shoots from five of six vines, whereas leaves from the middle and apical shoots yielded the viral antigens in only two vines. Roots of these same field-grown vines and leaves of greenhouse-grown vines were also sampled, and the viral antigens were detected in all of the vines (Table 1).

Stage 21: early flowering (38 days after budbreak). Once the viral antigens were detected consistently in the leaves, they were systematically and acropetally sampled to determine the rate of spread in the shoots. The viral antigens were detected in the apical, fully expanded leaf (leaf 6) of all vines and in the youngest expanding leaf (leaf 8) of three of the eight field vines. The highest concentration of viral antigens was found in the basal leaves (leaves 1–3) (Fig. 3, Table 2).

Stage 27: fruit set (45 days after budbreak). At this stage, the viral antigens remained apparently stationary in the basal leaves and were not consistently detected in the younger leaves (leaves 8–15) in the field. They were also detected in all flower samples. The flowers had dark anthers with ovaries starting to swell. Each sample of the most basal tendril of all vines gave a positive ELISA reading (Table 3).

Stage 29: berries groat-sized (55 days after budbreak). Unlike flowers, berries contained no detectable viral antigens, and viral antigens again were consistently found in basal but not in younger leaves (Table 3).

Stage 31: berries pea-sized (65 days after budbreak). Viral antigens were detected in younger leaves but did not reach the terminal leaves in five of the eight vines. They were detected in fruit and fruit pedicels in all cases. Tendril and bark samples of all eight field vines were positive for viral antigens (Table 3). On this date, an extra shoot of every vine was sampled, and the distribution was similar to that just described, with the exception of just one vine, in which leaf samples taken from the extra shoot were negative, although its fruit, tendril, and bark samples were positive. Initial symptoms of rolling and reddening started to appear in some leaves, but there was no

correlation between absence of leaf symptoms and lack of virus detection by ELISA. Leaves with and without symptoms from diseased vines showed positive ELISA readings.

Stage 32: developing berry (75 days after budbreak). Three shoots (basal, middle, and apical) were sampled from each vine in the field, and viral antigens were detected in all of them as well as in all root and in five of eight fruit samples. Basal leaves of any shoot consistently gave positive reactions. Table 3 shows data from basal shoots at leaf positions 3–8. This was confirmed by sampling three leaf positions (3, 8, and 15, where leaf 3 was the oldest) from each of the 11 shoots from one cane of one vine. Virus was detected in 11/11 samples of leaf 3, in 8/11 of leaf 8, and in 5/11 of leaf 15.

Stage 33: beginning of berry touch (85 days after budbreak). Three new shoots (basal, middle, and apical) were sampled from each vine, and the viral antigens were detected in every sample that was taken from leaves of each shoot, including the apical expanded leaf, except in the apical shoot of one of the eight field vines. The fruit, still green, was positive by ELISA in five of the eight vines mentioned in Table 3.

Stage 35: veraison, or beginning of ripening (105 days after budbreak). Leaves from one middle shoot of each of the test vines were assayed. Viral antigens were consistently detected in all sampled leaves (except the apical leaf of one vine). For the eight vines mentioned in Table 3, green fruits were positive by ELISA in four of the eight samples, whereas the darker, more mature fruits from the same cluster were all ELISA-positive.

Viral antigens were found in basal leaves with and without symptoms collected from basal, middle, and apical shoots of two canes of four infected vines. No differences in ELISA readings were found between symptomatic and symptomless leaves (Table 4).

Stage 36: berries ripening (120 days after budbreak). Viral antigens were detected in leaf samples taken at every fifth node including the youngest expanded leaf in all vines. Fruit samples from three of eight vines were ELISA-positive. Tendril samples from vines in Table 3 were all ELISA-positive.

Stage 38: berries ripe for harvest (128 days after budbreak). Viral antigens were found up to and including the youngest expanded leaf in the eight vines and also in the fruit samples, with the exception of one vine, in which the fruit cluster sampled showed uneven coloring and size of the berries. Viral antigens were not detected in the green, small, and abnormally immature fruits (Table 3).

Stage 40: harvest (153 days after budbreak). No new leaves had formed and the viral antigens were still detected up to and including the youngest expanded leaf and also in fruit and bark

Table 2. Optical density readings (A_{405nm}) of basal (1) to apical expanding (8) leaf samples from eight field-grown, leafroll-diseased Pinot noir grapevines at the time of early flowering (phenological stage 21) during 1986 measured by direct enzyme-linked immunosorbent assay using NY-1 antiserum prepared against closteroviruslike particles from these same diseased Pinot noir grapevines

Leaf position number	Vine										
	1	2	3	4	5	6	7	8	Mean	−CK[a]	+CK[a]
1	···	0.781	0.390	0.989	0.446	1.208	0.805	0.217	0.691		
2	0.347	0.610	0.460	0.616	0.659	0.763	0.775	0.290	0.564		
3	0.368	0.472	0.485	0.867	0.471	0.880	0.938	0.281	0.595		
4	0.231	0.243	0.139	0.576	0.295	0.493	0.515	0.235	0.341		
5	0.092	0.173	0.182	0.296	0.252	0.166	0.360	0.125	0.206		
6	0.065	0.052	0.101	0.123	0.252	0.078	0.146	0.061	0.110		
8	0.035	0.040	0.060	0.033	0.058	0.037	0.065	0.031	0.045	0.001	1.031

[a] Basal leaf samples from healthy (−CK) and leafroll-diseased (+CK) greenhouse-grown Pinot noir vines.

Table 3. Mean optical density readings ($A_{405\ nm}$) at different phenological stages of leaf samples from eight leafroll-diseased Pinot noir grapevines growing in the field during 1986, measured by direct enzyme-linked immunosorbent assay using NY-1 antiserum prepared against closteroviruslike particles from these same diseased Pinot noir field vines

Tissue sampled and leaf position number	Mean optical density at phenological stage[a]								
	Fruit set	Groat-sized	Pea-sized	Developing	Touch	Veraison	Ripening	Ripe	Harvest
3	0.759 ± 0.241[b]	···	1.031 ± 0.289	1.196 ± 0.260	···	···	···	···	···
5	···	0.781 ± 0.268	···	···	···	···	0.786 ± 0.166	···	0.870 ± 0.231
8	0.029 ± 0.002	0.128 ± 0.082	0.414 ± 0.291	0.681 ± 0.371	1.500 ± 0.000	···	···	···	···
10	0.052 ± 0.004	0.008 ± 0.016	0.256 ± 0.119	···	1.300 ± 0.212	···	0.894 ± 0.160	···	0.721 ± 0.230
15	0.021 ± 0.012	0.006 ± 0.012	0.209 ± 0.214	0.194 ± 0.231	1.090 ± 0.301	···	0.927 ± 0.249	···	···
20	···	···	0.052 ± 0.045	0.032 ± 0.025	0.647 ± 0.557	1.467 ± 0.088	0.822 ± 0.172	0.862 ± 0.263	0.770 ± 0.249
25	···	···	···	0.006 ± 0.010	0.156 ± 0.110	1.280 ± 0.366	0.699 ± 0.152	0.698 ± 0.187	0.733 ± 0.220
30	···	···	···	0.000	0.090 ± 0.070	0.612 ± 0.410	0.406 ± 0.211	0.754 ± 0.245	0.595 ± 0.132
35	···	···	···	···	···	0.198 ± 0.030	0.216 ± 0.110	0.208 ± 0.290	0.606 ± 0.079
40	···	···	···	···	···	0.087 ± 0.032	0.083 ± 0.010	0.170 ± 0.052	0.469 ± 0.121
Tendril	0.374 ± 0.113	···	0.090 ± 0.035	···	···	···	0.194 ± 0.128	···	···
Bark	···	···	0.261 ± 0.120	···	···	···	···	···	0.925 ± 0.267
Root	···	···	···	0.107 ± 0.044	···	···	···	···	···
Fruit	0.594 ± 0.136	0.006 ± 0.016	0.130 ± 0.123	0.104 ± 0.094	0.063 ± 0.035	0.130 ± 0.049[c]	0.050 ± 0.043	0.128 ± 0.053	0.182 ± 0.052
	···	···	···	···	···	0.073 ± 0.69[d]	···	···	···
−CK[e]	0.002	0.000	0.003	0.000	0.003	0.000	0.000	0.002	0.001 ± 0.002

[a] Following the phenological system of von Eichhorn and Lorenz (25).
[b] Mean optical density ($N = 8$) ± standard deviation. All ELISA readings for unknowns exceeding 0.050 were rated as positive.
[c] Dark, ripening berries.
[d] Green berries from the same cluster.
[e] Basal leaf samples from healthy Pinot noir vines growing in the greenhouse.

samples.

Internal spread of the virus during a growing season. The distribution of viral antigens in leaves along infected shoots was determined with data from all sampling dates (Tables 1–3). Total number of leaves at each sampling date and leaf position number of the youngest infected leaf of each selected shoot were recorded, and averages of the eight vines from the field are presented in Figure 4.

DISCUSSION

GLR-associated particles have been reported in New York as phloem-restricted. They were purified, and a highly specific antiserum was developed (26).

We found that bark extracts of dormant cuttings collected from diseased vines in the field and maintained at 6 C for 6 mo gave ELISA-positive reactions. After harvest and defoliation, the virus probably overwinters in the phloem of canes and, though not tested, perhaps also in the phloem of roots. Other investigators have found the bark tissue of dormant canes to be good sources for electron microscope observation of closterovirus associated with GLR disease (5,15,16) or for the extraction of its double-stranded RNA (2).

As soon as flower clusters were formed and exposed, they and the roots became the best tissues for the earliest detection of the viral antigens (Fig. 1, Table 1). This could probably be explained by considering that the initial movement of this virus follows a source-to-sink pattern (8). In this case, the tissue source could be the cane bark, and the sink of assimilates could be the flowers and roots. We postulate that this virus overwinters in the phloem of canes and roots, and as soon as vegetative growth is reinitiated, it moves to flower clusters and new roots. Root extracts have been used successfully to detect, with the electron microscope, a closterovirus in grapevines indexing positive for GLR by bioassay (9).

At the beginning of vegetative growth, viral antigens were not detected in leaves (Figs. 1 and 4, Table 1). We wanted to determine the rate of movement, expecting spread from basal to apical leaves, but when the systematic and acropetal sampling of leaves was made (at early flowering, 38 days after budbreak), the viral antigens were already detected up to and including the youngest expanded leaf (Fig. 3, Table 2). Future work to determine the rate of detection in leaves of growing shoots would require more frequent sampling from the time when the inflorescence is fully developed until early flowering.

There appeared to be three periods of viral antigen movement in the vine as measured by progressive infection of the shoots in the field: 1) from budbreak to inflorescence swelling, viral antigens were apparently restricted to the flower clusters and were not detected in leaves; 2) from inflorescence fully developed to developing berries (25–75 days after budbreak), viral antigens were detected in the leaves but did not reach the terminal ones; and 3) at the beginning of berry touch stage (85 days after budbreak), viral antigens were detected up to and including the terminal leaf and remained in that position until harvest (Fig 4, Tables 1–3).

In general, viral antigens showed an even distribution in the vines (Fig. 2), which contrasts with the uneven detection of tomato ringspot virus in grapevines (11,12) or in peaches (1). The regular distribution of these viral antigens may be explained by their association with phloem tissues and the movement of assimilates throughout the vine.

There was a consistent difference in apparent viral antigen concentration (Table 1) and likelihood of detection (Table 4) when the leaf samples were taken from basal nodes as compared with

Table 4. Detection of viral antigens during 1986 in basal leaves with (W) and without (W/O) symptoms of leafroll collected from basal (B), middle (M), and apical (A) shoots of two canes (C1 and C2) of four leafroll-disesased Pinot noir grapevines growing in the field, at the beginning of ripening (phenological stage 36), as measured (A_{405nm}) by direct enzyme-linked immunosorbent assay using NY-1 antiserum prepared against closteroviruslike particles from these same diseased Pinot noir field vines

Leaf source, position, and symptoms	Pinot noir grapevine number				Mean
	1	2	3	4	
C1 B W	1.5[a]	1.5	1.5	1.488	1.497
C1 B W/O	1.5	1.5	1.5	1.500	1.500
C1 M W	1.5	1.5	1.5	1.500	1.500
C1 M W/O	1.5	1.5	1.5	1.500	1.500
C1 A W	1.5	1.5	1.5	1.482	1.496
C1 A W/O	1.5	1.5	1.38	1.500	1.470
C2 B W	1.5	1.5	···	0.513	1.171
C2 B W/O	1.5	1.5	···	1.500	1.500
C2 M W	1.5	1.5	···	1.409	1.470
C2 M W/O	1.5	1.5	···	1.500	1.500
C2 A W	1.5	1.5	···	1.442	1.481
C2 A W/O	1.5	1.5	···	0.588	1.196
−CK[b]	0.000	0.003	0.000	0.001	0.001

[a] ELISA reader upper detection limit = 1.5. All ELISA readings for unknowns exceeding 0.050 were rated as positive.

[b] −CK = leaf extracts from healthy Pinot noir vines growing in a greenhouse.

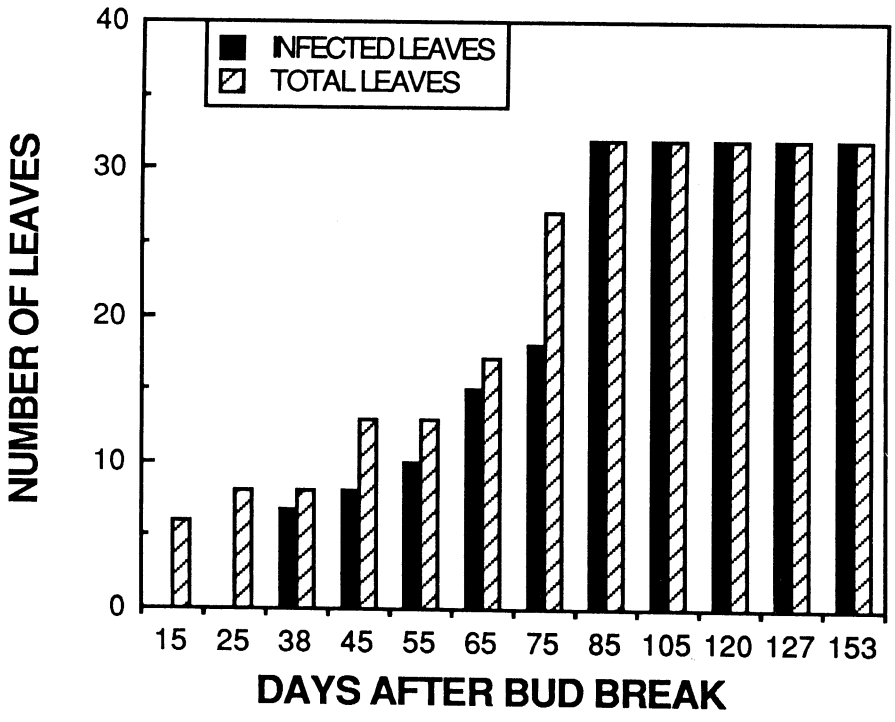

Fig. 4. Average total number of leaves and number containing viral antigens in the youngest leaf of one shoot from each of eight leafroll-diseased Pinot noir vines in the field at different days after budbreak. Viral antigens were not detected in leaves during the first 15 days after budbreak. From 25 days (inflorescence fully developed) to 75 days (developing berry) after budbreak, viral antigens were found in leaves but not in the terminal ones. From berry touch (85 days) until harvest (153 days after budbreak), viral antigens were detected up to and including the terminal leaf.

more terminal nodes. This is important for large-scale sampling to increase the frequency of detection of these viral antigens or even for collection of leaf samples for direct observation under the electron microscope. It is important to emphasize the localization of viral antigens in apparently higher concentrations in basal than in apical leaves (Fig. 3, Tables 1–3). Many other viruses are more frequently found in apical leaves, and that could explain the inconsistent results obtained in various attempts to observe the GLR-associated particles under the electron microscope in young tissues (3,6,7,10,22).

It is interesting to note that viral antigens were detected in symptomatic and symptomless leaves in diseased but never in healthy vines (Table 4). For as long as basal leaves were sampled, viral antigens were detected, beginning 2 mo before foliar symptoms appeared (Tables 1–3).

The detection of this virus in the bark of dormant canes would facilitate the assay of cuttings interchanged between different places. The replacement of the traditional grafting in woody indicators by serological detection would reduce the time of assay from 2 yr to 2 days; labor and space required would be reduced, and the number of tests could be increased. The apparent concentration of viral antigens in bark of dormant canes or of growing shoots (Table 1) is high and reliable for detection purposes. GLR might be caused by more than one virus (3,5,13,15) and by different strains of one virus. Direct ELISA (4) is so specific that, for many plant viruses, it fails to detect other serologically closely related strains (14,18). Therefore, a broader system that will detect leafroll isolates by means of serology has to be carefully considered. Work in this regard is under way.

Pathogenicity tests with this closterovirus are in progress. This is important because there is no general acceptance of the role of the various closteroviruses (including NY-1) that have been found associated with GLR disease. Koch's postulates must be carefully followed: postulate 1 (constant association) requires serious consideration to avoid confusion between sporadic or casual findings and repeatable, demonstrable constant association. Special attention must be given to the healthy negative checks in ELISA (19) and in electron microscope work so that particles are found only in diseased plants and not in healthy ones, as has been sometimes reported (7,21,24).

In conclusion, our results show that during the 1986 grapevine-growing season in Seneca County, NY, GLR-associated viral antigens were detected in eight vines from one field as soon as the flower clusters were exposed and in the roots, 2 mo before any typical disease symptom appeared on leaves. Healthy greenhouse-grown Pinot noir vines were included as controls in all samplings and always were ELISA-negative. Leaves, which are most frequently used for serological detection or for electron microscope observation, were not reliable viral antigen sources until bloom. The viral antigens were evenly distributed in the canes produced the previous year. From the time of flowering, viral antigens were consistently detected in the basal leaves of any shoot. The rate of advance of the viral antigens in the leaves of growing shoots apparently followed a three-period schedule. Bark phloem of dormant or growing canes was a good source for viral antigen detection by ELISA. We believe that these results may be useful to researchers working with the etiology, detection, and control of this important disease.

ACKNOWLEDGMENTS

We wish to thank David Hummer and Eunice Williams for technical assistance. The work was supported by BARD (US-1036-85), N.Y. State Agricultural and Markets, N.Y. Grape Production Research Fund, Colegio de Posgraduados, and CONACYT, Mexico.

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