Symptom Expression and Disease Occurrence of a Yellows Disease of Grapevine in Northeastern Italy

R. OSLER, Professor, L. CARRARO, Researcher, N. LOI, Researcher, and E. REFATTI, Professor, University of Udine, Institute of Plant Protection, Udine 33100, Italy

ABSTRACT

Osler, R., Carraro, L., Loi, N., and Refatti, E. 1993. Symptom expression and disease occurrence of a yellows disease of grapevine in northeastern Italy. Plant Dis. 77:496-498.

A severe yellows type disease of grapevine (GYD) has been detected in various regions of Italy since 1982. One of the most susceptible cultivars is Chardonnay. Symptoms of GYD are indistinguishable from those described for flavescence dorée (FD) in France. In northeastern Italy, the spread of GYD is not correlated with the presence of the leafhopper *Scaphoideus titanus,* a vector of FD. A 6-yr trial was carried out to acquire information on the epidemiology of GYD in northeastern Italy and on the effect of GYD infection on grapevines. The presence of an active vector(s) was demonstrated by exposing young, healthy grapevines, cultivar Chardonnay, to natural inoculations. The minimum incubation period for GYD did not exceed 5 mo. Infected Chardonnay grapevines protected in an insect-proof plastic screenhouse showed a transitory recovery (i.e., remission of symptoms followed by symptom reappearance). The length of the symptomatic period was quite variable when it was not influenced by reinfection; and the plants protected from reinfection gradually, but not completely, recovered.

Several diseases of grapevines (*Vitis vinifera* L.) ascribed to prokaryotic organisms are reported to occur in Europe and elsewhere. Among them are flavescence dorée (FD) and other grapevine yellows diseases (GYD) caused by mycoplasmalike organisms (MLOs) (4,11, 15,18).

FD was detected and described for the first time in France (3), and the causal agent was experimentally transmitted by the leafhopper *Scaphoideus titanus* Ball (formerly *Scaphoideus littoralis* Ball) (20). *S. titanus* is considered the natural vector of FD.

In Italy, the first cases of an FD-type disease (based on symptom expression) were reported in Lombardy (2). The causal agent in this region has been experimentally transmitted from grapevine to grapevine using the vector *S. titanus* (10).

After the first Italian report of the FD-type disease, a severe yellows-type disease caused by an MLO (1,13) occurred in several regions of Italy on different grapevine cultivars (8,12,19). Chardonnay is the most susceptible among the more important cultivars. The symptoms of GYD are indistinguishable from the symptoms of FD in France. Since an epidemic phase of GYD, the disease has decreased and does not appear to be correlated with populations of *S. titanus* in vineyards (14,17). Whether insecticides can be used to control the disease in the northeastern part of Italy has not been determined (9,17).

The responses of grapevines to GYD are not uniform. Grapevines of the same cultivar in a field can alternate symptomatic and asymptomatic phases. In areas where potential vectors are active, it is impossible to determine whether the reappearance of symptoms in a previously "recovered" grapevine is due to reinfection or to the end of a transitory remission period. Similarly, we do not know whether recurrent infections are necessary for the same grapevine to show symptoms in consecutive years.

In this study, we compared the behavior of two groups of infected grapevines, one transplanted under an insect-proof plastic screen, the other freely exposed to natural inoculations, to understand the influence of reinfections on the length of the symptomatic period and on recovery or transitory recovery. By exposing young, healthy grapevines to natural infections for six consecutive years, we hoped to verify the presence of active vectors, to measure the local infection pressure, and to calculate the incubation period of the disease. In addition, a 6-yr disease-incidence survey was carried out in representative vineyards of the Friuli–Venezia Giulia (F-VG) to discover the progress of the disease in this important grape-growing region of Italy.

MATERIALS AND METHODS

Development and remission of symptoms in transplanted grapevines. In spring 1987, 150 3-yr-old Chardonnay grapevines that had shown typical symptoms of GYD the previous year were rogued from a commercial vineyard in the Casarsa area (F-VG region) that contained about 10% symptomatic plants. The vines were pollarded and planted in a field in the same area. Fifty

Accepted for publication 30 November 1992.

plants were covered with an insect-proof plastic screen (a screenhouse about 2.5 m high) and treated with insecticides every 10 days from April to October. The remaining 100 grapevines were not covered.

Exposure of healthy young grapevines to natural inoculations. In 1986, 500 young virus-free Chardonnay grapevines grafted onto the rootstock Kober 5BB were obtained from the Trento grape area. GYD had not been reported in this area. In spring 1987, 200 of these grapevines were brought to the Casarsa experimental field (described above). A total of 100 grapevines were planted in the open field, and 100 were planted in the same field inside a screenhouse. The remaining 300 were equally divided and grown in three insect-proof screenhouses erected near Udine, Trento, and Rovigo, and used as additional healthy controls. The protective plastic screen was renewed every 1–2 yr.

Progress of the disease in the F-VG region. For 6 yr (1987–1992), about 15,000 Chardonnay grapevines in 15 representative vineyards of the F-VG region were visually checked to determine the natural progress of the disease on a large scale under field conditions.

Every plant was checked at least three times per year (from June 1987 to October 1992). The infectivity of the test plants was based on symptom expression, because antiserum was not available. The GYD syndrome on Chardonnay is diagnostic, and this cultivar is a specific indicator host for the disease. The diagnosis was based on the contemporaneous presence in a single plant of at least three of the following symptoms: leaf yellowing and sectorial discolorations; crisp, stiff, downward-cupped leaves with a thick lamina showing characteristic necrotic areas; limp and rubbery shoots; incompletely matured canes with dark pustules concentrated at the base; and aborted fruit clusters and dried rachis, berries, and tendrils.

In all the screenhouses and vineyards under investigation, the leafhopper populations were periodically examined. Monitoring of the hopper population was done with yellow sticky traps (11.5 × 21 cm) renewed weekly or fortnightly from April to October, and by capturing insects with entomological nets.

RESULTS

Development and remission of GYD symptoms in the transplanted grapevines. The results of the 6-yr survey of the two groups of grapevines transplanted from the original vineyard are shown in Fig. 1. In the year of transplantation (1987), the percentage of symptomatic grapevines decreased similarly both in exposed (35%) and protected (34%) groups of plants. The following years, the percentage of symptomatic grapevines was higher in the group of exposed plants. The percentage of exposed grapevines with GYD symptoms was 48% in 1988, 58% in 1989, 49% in 1990, 52% in 1991, and 58% in 1992. For protected plants, the percentages were 20% in 1988, 28% in 1989, 28% in 1990, 24% in 1991, and 28% in 1992.

Table 1 shows the annual number and percentage of grapevines that ceased to exhibit GYD symptoms ("recovered") after having shown them the previous year.

Among the protected grapevines–where no reinfection took place–four plants out of 50 exhibited symptoms for six consecutive years, one for 5 yr, five for 4 yr, four for 3 yr, five for 2 yr, and six for only 1 yr; 25 plants (50%) appeared healthy during the entire 6-yr period after transplanting. (In the exposed group, 29 plants [29%] remained symptomless during the 6-yr period.)

Among the protected plants that temporarily recovered, one showed symptoms again after being asymptomatic for 3 yr, three after 2 yr, and seven after 1 yr.

Exposure of healthy young grapevines to natural inoculations. The number of symtomatic plants among the originally healthy grapevines exposed to natural inoculations in the Casarsa experimental field is shown in Table 2. Up to early October 1987, four of the grapevines exhibited typical symptoms of the disease; during the following years, the number showing symptoms increased. During the 6 yr of exposure, 30 plants developed symptoms. The number of plants that exhibited symptoms for the first time during the 1988–1992 seasons were respectively 11, 8, 2, 2, and 3; the number of recovered plants were 2, 5, 1, 4, and 2.

None of the 100 healthy grapevines grown in the screenhouse of Casarsa exhibited symptoms of GYD during the 6 yr of the experiment. The same was true for the other 300 grapevines planted in the screenhouses of Udine, Trento, and Rovigo.

The hoppers species *Empoasca vitis* (Gothe), *Euscelis incisus* (Kirshbaum), *Euscelidius variegatus* (Kirshbaum), *Macrosteles cristata* (Ribaut), *M. laevis* (Ribaut), *M. quadripunctulatus* (Kirshbaum), *Metcalfa pruinosa* Say, *Philaenus spumarius* (L.), *S. titanus, Zygina rhamni* Ferrari, and *Zyginidia pullula* Boheman were captured each year in the

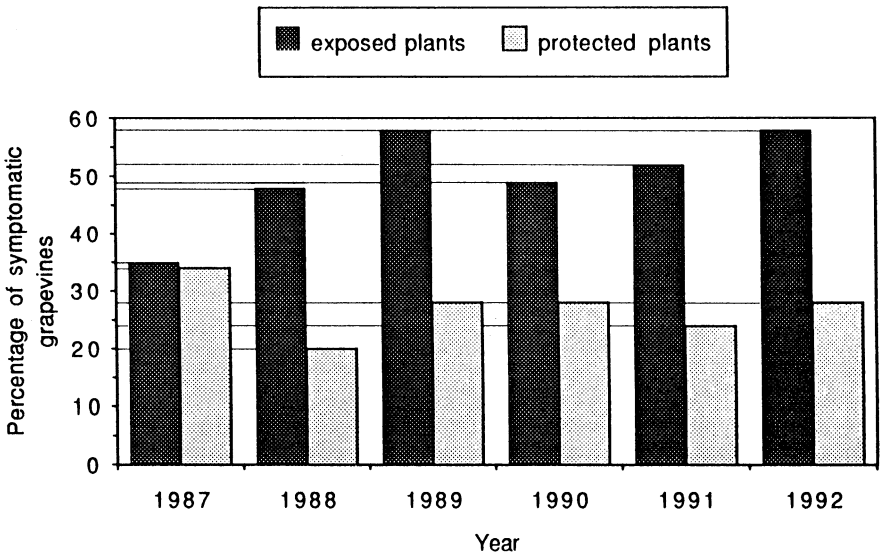

Fig. 1. Symptoms on two groups of affected grapevines surveyed for 6 yr after transplanting. In spring 1987, 150 grapevines (Chardonnay) that had exhibited symptoms of grapevine yellows disease during the previous year were rogued from the original vineyard and transplanted to the Casarsa experimental plots, and either protected under an insect-proof screenhouse or exposed to natural inoculations.

Table 1. Number and percentage of affected grapevines transplanted in 1987 (as seen in Fig. 1), that ceased to exhibit grapevine yellows disease symptoms after having shown them the previous year

	Protected plants		Exposed plants	
Year	(no.[a])	(%)	(no.[a])	(%)
1987	33/50	66	65/100	65
1988	8/17	47	11/35	31
1989	2/10	20	5/48	10
1990	1/14	7	10/58	17
1991	3/14	21	4/49	8
1992	1/12	8	0/52	0

[a] Plants that ceased to exhibit symptoms (numerator) divided by plants that were symptomatic the previous year (denominator).

Table 2. Symptoms on the 100 originally healthy grapevines exposed to natural inoculation in the experimental field of Casarsa. The corresponding 100 plants protected under plastic screen never showed symptoms

Year	Symptomatic plants	Plants that showed symptoms for the first time	Plants that recovered after showing symptoms the previous year
1987	4	4	...
1988	13	11	2
1989	16	8	5
1990	21	2	1
1991	21	2	4
1992	24	3	2

experimental field of Casarsa. Among the occasionally ampelophagous species, only the *Z. pullula* was almost constantly present. A maximum of 100 *Z. pullula* in 1987 and a minimum of 29 in 1988 were captured in the four sticky traps. Captures of the other species of hoppers were sporadic. Analogous data were obtained in the commercial vineyards of the F-VG region. Among the ampelophagous hoppers captured in the Casarsa experimental field, the *S. titanus* population was modest (fewer than 10 individuals per year). The population of *E. vitis* (5,120 individuals in 1988) in this field was very high. Transmission trials of the GYD agent from grapevine to grapevine by *S. titanus* and other species of leafhoppers are in progress, and the results indicate *S. titanus* is not an efficient vector under our conditions (16).

Progress of the disease in the F-VG Region. The average percentages of Chardonnay grapevines showing GYD symptoms in the 15 vineyards checked during 1987–1992 were, respectively, 11, 13, 13, 12, 11, and 10%; the corresponding increment indices (annual percentage of newly infected plants divided by the percentage of recovered ones) were 2.8, 2.3, 1.0, 0.9, 0.7, and 0.8. The incidence of the disease in the single vineyards varied from 1 to 49%; the increment of the symptomatic plants ranged in the 6-yr period from 0 to 130%.

DISCUSSION

The responses of affected grapevines transplanted to plots in an open field without protection were variable, ranging from no recovery to temporary or permanent recovery. The high rate of apparent recovery that occurred the first year after transplantation was exceptional in both protected and exposed plots. This phenomenon may be due to the cumulative effects of root reduction and pollarding. The recovery rate of the corresponding affected plants pollarded but not transplanted from the original vineyard was 12%, versus 65% for both pollarding and transplanting. From the second to the sixth year after transplanting, the percentages of symptomatic plants of the exposed group and the screenhouse-contained group became quite different. Considering the proven activity of natural vectors occurring in the experimental area, the differences during the last 5 yr of the trial are likely due to natural infections. The recovery phenomena occurred both inside and outside the screenhouse, and in the open field a certain amount of recovery was prevented by new infections.

Some of the results obtained from the originally infected grapevines grown inside the screenhouse of the Casarsa experimental field lead to the conclusion that the length of the symptomatic period is variable even when it is not influenced by reinfections. It was also proved that in Chardonnay a symptomatic period exceeding one year is not necessarily the result of successive reinoculation, and that transitory recovery (remission followed by symptom reappearance) is not always due to reinfections, as shown in France for the hybrid Baco 22A and other cultivars affected by FD (5,6,7).

The results with originally healthy young grapevines exposed to natural infections indicated that in the Casarsa area at least one MLO vector is present. Because in 1987 the first symptoms appeared in early October, the minimum incubation period, if the inoculation is due to insects, does not exceed 5 mo. Except for 1991, the annual number of plants that appeared to recover was lower than the number with new infections and/or reinfections, which explains the progressive increase in the number of affected grapevines year by year.

The data obtained from the surveys of 15 vineyards of the F-VG region indicate that the dissemination of the disease gradually decreased in this area. Nevertheless, it continues to spread and is characterized by recoveries and renewed infection.

In Italy, the true role of *S. titanus* in the transmission of GYD is not known. This research is being continued to verify the importance of this leafhopper and alternative species as vectors of GYD.

ACKNOWLEDGMENTS

We thank T. A. Chen for his review of the paper and F. Pavan for the identification and monitoring of the leafhoppers. The research is supported by the Italian Ministry of Agriculture and Forestry (Extraordinary Programme of Research on Grapevine Flavescence Dorée).

LITERATURE CITED

1. Arzone, A., Alma, A., Patetta, A., and Bosco, D. 1991. Grapevine golden flavescence MLOs in plant and vector. Pages 184-192 in: Proc. Meet. Int. Counc. Study Viruses Virus Dis. Grapevine, 10th. I. C. Rumbos, R. Bovey, D. Gonsalves, W. B. Hewitt, and G. P. Martelli, eds. ICVG.
2. Belli, G., Fortusini, A., Osler, R., and Amici, A. 1973. Presenza di una malattia del tipo "flavescence dorée" in vigneti dell'Oltrepò pavese. Riv. Patol. Veg., S. IV, 9:49-56.
3. Caudwell, A. 1957. Deux années d'études sur la flavescence dorée, nouvelle maladie grave de la vigne. Ann. Amélior. Plant. 4:359-393.
4. Caudwell, A. 1961. Etude sur la maladie du bois noir de la vigne: ses rapports avec la flavescence dorée. Ann. Epiphyt. 12:241-262.
5. Caudwell, A. 1961. Les phénomènes de rétablissement chez la flavescence dorée de la vigne. Ann. Epiphyt. 12:347-354.
6. Caudwell, A. 1964. Identification d'une nouvelle maladie à virus de la vigne, la flavescence dorée. Etude des phénomenès de localisation des symptomes et de rétablissement. Ann. Epiphyt. 15 (hors-ser. 1), 193 pp.
7. Caudwell, A. 1981. La flavescence dorée de la vigne en France. Phytoma n325:16-19.
8. Credi, R. 1989. Flavescenza dorata della vite in Emilia-Romagna: evoluzione della malattia nelle piante e suoi effetti sulla produzione e sullo sviluppo vegetativo. Phytopathol. Mediterr. 28:113-121.
9. Fortusini, A. Pontiroli, R., and Belli, G. 1988. Nuovi dati e osservazioni sulla flavescenza dorata della vite nell'Oltrepò pavese. Vignevini 15(3):67-69.
10. Fortusini, A., Saracchi, M., and Belli, G. 1989. Trasmissione sperimentale della flavescenza dorata della vite mediante *Scaphoideus titanus* Ball. in Italia. Vignevini 16(9):43-46.
11. Gaertel, W. 1965. Untersuchungen ueber das Auftreten und das Verhalten der Flavescence dorée in den Weinbaugebieten an Mosel und Rhein. Weinberg & Keller 12:347-376.
12. Granata, G. 1982. Deperimenti e giallume in piante di vite. Inf. Fitopatol. 32(7-8):18-20.
13. Granata, G., and Grimaldi, V. 1991. Electron microscopic detection of mycoplasma-like organisms in epidemic yellow affected grapevines. Petria 1(3):171-176.
14. Granata, G., and Russo, A. 1990. Indagini su un giallume epidemico simile alla "flavescenza dorata." Vignevini 17(5):69-71.
15. Magarey, P. A., Plavsic, B., and Wachtel, M. F. 1988. Mycoplasmalike organisms associated with Australian grapevine yellows. Plant Dis. 72:363.
16. Osler, R., Boudon-Padieu, E., Carraro, L., Caudwell, A., and Refatti, E. 1992. First results of the trials in progress to identify the vector of the agent of a grapevine yellows in Italy. Phytopathol. Mediterr. 31:175-181.
17. Pavan, F. 1989. Possibilità di controllo dei potenziali vettori dell'agente della flavescenza dorata. Inf. Agrario 41:55-61.
18. Pearson, R. C., Pool, R. M., Gonsalves, D., and Goffinet, M. C. 1985. Occurrence of flavescence dorée-like symptoms on "White Riesling" grapevines in New York, U.S.A. Phytopathol. Mediterr. 24:82-87.
19. Refatti, E., Carraro, L., Pavan, F., Osler, R., and Girolami, V. 1988. La flavescenza dorata della vite. Not. E.R.S.A. 1(2):1-16.
20. Schvester, D., Carle, P., and Moutous, G. 1963. Transmission de la flavescence dorée de la vigne par *Scaphoideus littoralis* Ball. Ann. Epiphyt. 14:175-198.