Scaphoideus titanus, a Possible Vector of Grapevine Yellows in New York

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ABSTRACT

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Scaphoideus titanus, the natural vector of grapevine *flavescence dorée* (FD), is widespread in New York. It was found closely associated with both wild *Vitis riparia* and cultivated *V. vinifera,* but it prefers *V. riparia.* Adult leafhoppers migrate from surrounding wild grapevines into the peripheral parts of vineyards. *V. vinifera* vines with symptoms of grapevine yellows (GY) disease were concentrated along the borders of vineyards. Even near GY-affected vineyards, *V. riparia* vines never exhibited symptoms of this disease. Symptoms of an MLO infection developed in 29% of *Vicia faba* plants fed on by field-collected *S. titanus.* However, potted *V. vinifera* 'Chardonnay' on which the same leafhoppers were fed did not develop symptoms of GY within 1 yr. Thirteen percent of 371 *S. titanus* leafhoppers collected from *V. vinifera* or *V. riparia* reacted positively in enzyme-linked immunosorbent assays (ELISAs) with polyclonal antibodies to FD, and MLOs were detected by immunosorbent electron microscopy in extracts of ELISA-positive leafhoppers. The results support the hypothesis of a common North American origin of FD and its vector, *S. titanus,* but further research is necessary to confirm the relationship of *S. titanus* and the pathogens found in this leafhopper to GY.

Yellows diseases of grapevine (*Vitis vinifera* L.) are reported from viticultural areas of Europe (3,6,20,31), North (30) and South (29) America, and Australia (25). These diseases are almost identical in symptomatology but differ with respect to susceptible cultivars and epidemiology. Shoots of affected vines lack periderm and exhibit an irregular pattern of lignification, together with black pustules along internodes. Shoot tips, tendrils, and inflorescences become necrotic, and leaves often curl downward, become yellow or red depending on the cultivar, and abscise prematurely. Berries shrivel and remain sour. Symptomatic and nonsymptomatic shoots often grow on the same vine. *Flavescence dorée* (FD) of southern France and northern Italy and some other yellows diseases in the Mediterranean area spread rapidly through vineyards, affecting almost 100% of an individual planting within a few years (9). Other yellows diseases, such as *Vergilbungskrankheit* of Germany and *bois noir* of northern France, are endemic but may occur at a high incidence (20).

Of all grapevine yellows (GY) diseases, FD has been most investigated because of its economic importance. It is caused by a mycoplasmalike organism (MLO) (10) that is transmitted naturally by a deltocephaline leafhopper, *Scaphoideus titanus* Ball (syn. *S. littoralis* Ball) (32). This leafhopper was introduced into France from North America (7). Neither pathogens nor vectors have been identified for the other GY diseases.

After the vector and pathogen of FD were identified, control strategies and diagnostic procedures were developed. The spread of FD in France is effectively suppressed by insecticide applications (8,16,28). The pathogen can be detected in plants and vectors by various serological procedures (4,5,15,23,24,33). Recently, DNA hybridization was successful in detecting the MLOs of FD in leafhoppers (19).

The close association of FD with *S. titanus,* together with the observation that American grape species and rootstocks can be infected by FD but develop no symptoms or only weak symptoms (7,27), led to the assumption that the pathogen, like its vector, originates from North America. Caudwell (7) described a hypothetical natural cycle of the pathogen of FD in North America between wild *Vitis* spp. that remain symptomless after infection and *S. titanus.* According to the hypothesis, the FD pathogen may have been introduced into Europe in wood of American *Vitis* spp. used in breeding programs, and some time later the vector, *S. titanus,* was introduced. Once introduced, it transmitted the FD pathogen to local *V. vinifera* cultivars, which, not being adapted to the pathogen, developed symptoms of FD.

Pearson et al (30) reported a yellows disease on *V. vinifera* 'White Riesling' in New York. The symptoms are identical to those of FD, but the disease occurs in low incidence and seems to spread slowly in vineyards. The occurrence of this disease in the area of presumed origin of *S. titanus* supports Caudwell's hypothesis.

In this study, we attempted to determine the distribution of *S. titanus* on grapevines in New York, to find possible relationships between this leafhopper and the North American GY disease, to evaluate the role of the common wild *Vitis* spp. in the epidemiology of GY, and to collect information about the possible relatedness of the North American GY disease and FD. A preliminary report of this research has been published (26).

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MATERIALS AND METHODS

Leafhopper collection and identification. Individual leafhoppers for transmission experiments were collected with an aspirator from leaves of wild (*V. riparia* Michx.) and cultivated grapevines growing at Valois and Dresden in the Finger Lakes region of New York from June to September 1989 and from May to August 1990. Both vineyards were planted with *V. vinifera* 'White Riesling' and 'Chardonnay', and hedgerows on the borders contained many *V. riparia.* Each site was sampled randomly twice a week for about 3 hr. At five commercial vineyards in Valois and Dresden, two yellow sticky traps were placed in the hedgerows, at the edge of the vineyards, and within the vineyards 25 or 50 m from the edge to collect leafhoppers for species identification and to gather information about the distribution of leafhoppers within vineyards. Single leafhoppers were collected with an aspirator at various locations in the Finger Lakes region, on Long Island, and near Westfield, NY, on Lake Erie.

The keys of Beirne (2) and Barnett (1) were used for species determination. Eggs detected in the bark of *V. vinifera* and *V. riparia* and nymphs collected in the field were reared to adulthood in the laboratory for species determination. Internal genitalia of male adult leafhoppers were prepared as described by Beirne (2).

Transmission experiments. Broad bean (*Vicia faba* L.) plants and potted Chardonnay grapevines were used as indicators in greenhouse transmission experiments. Leafhoppers collected from wild or cultivated grapevines were kept in groups of 15 on broad beans for 3 days and then on grapevines for 30 days in nylon mesh cages attached to the pots. Plants and insects were kept in light for 16 hr daily at 25 C and in darkness for 8 hr at 18 C. After the transmission period, all insects were removed from the plants and stored in 50 μl of 10 mM phosphate-buffered saline (PBS), pH 7.4, at −20 C for future serological tests (4).

Enzyme-linked immunosorbent assay (ELISA). Leafhoppers that survived the transmission period in the greenhouse were used for ELISA at Geneva, NY. All antisera, infected leafhopper standards, and laboratory-bred, healthy leafhoppers were produced at Institut national de la recherche agronomique in Dijon, France.

An indirect double-antibody sandwich ELISA procedure was used for all leafhopper tests. Individual leafhoppers were ground in a final volume of 500 μl of 10 mM PBS (pH 7.4) and centrifuged, and the clear supernatant was retained for testing. Antibodies produced to antigens isolated from FD-infected bean tissue were used to avoid nonspecific reaction with leafhopper antigens (4). Microtitration plates (Dynatech Immulon) were coated with polyclonal rabbit anti-FD immunoglobulin G (IgG) at a final concentration of 4 μg/ml for 4 hr. All steps except antigen binding were performed at room temperature. Blocking was done with Tris-casein buffer for 2 hr. Antigen binding was allowed overnight at 4 C. Mouse anti-FD IgG (final concentration 7 μg/ml) was used for 4 hr as the second antibody. Alkaline phosphatase-conjugated goat antimouse IgG (SIGMA A-7659) was used at a dilution of 1:1,000 for 2 hr, and *p*-nitrophenylphosphate (SIGMA N-9389) was added as substrate (1 mg/ml). Absorbance at 405 nm (A_{405nm}) was recorded after 90 min with a Dynatech MR 580 reader. On each plate, 30 field-collected and five laboratory-bred, healthy *S. titanus* were examined in two replications with 100 μl of extract per well together with infected standards. The standards were prepared in Dijon by extraction of a group of FD-infected leafhoppers as described above and dilution of the extract 1:3, 1:9, 1:27, 1:80, and 1:240 (v/v) with extracts of laboratory-bred, healthy leafhoppers.

A positive-negative threshold was calculated as the mean of A_{405nm} values of healthy leafhoppers on each plate plus three times the standard deviation (35). ELISA responses of leafhoppers of different sex and developmental stage were compared with respect to their collection site and host plant by Wilcoxon rank-sum tests (SAS/STAT software package, SAS Institute, Cary, NC).

Immunosorbent electron microscopy (ISEM). Samples from seven field-collected, ELISA-positive leafhoppers and four laboratory-bred, healthy leafhoppers were taken from extracts prepared for ELISA. Copper electron microscope grids were coated with Formvar and incubated with Protein-A (SIGMA P-6031) at 10 μg/ml for 10 min, then coated with mouse anti-FD IgG (7 μg/ml) for 5 min. Antigen binding was allowed by floating the grids on leafhopper extracts at 4 C overnight. Decoration was accomplished with mouse anti-FD IgG (35 μg/ml) for 10 min. PBS (pH 7.4) was used for rinsing. The samples were stained with 1.5% phosphotungstic acid after fixation with 1% glutaraldehyde for 2 min and rinsing with distilled water. The samples were examined with a JEOL JEM-100 SX transmission electron microscope.

RESULTS

Leafhopper survey. *S. titanus* was the most common deltocephaline leafhopper on grapevines in New York. In the Finger Lakes region, leafhoppers were collected from the west side of Cayuga Lake to the west side of Keuka Lake. In this area, *S. titanus* was found on wild *V. riparia* in hedgerows at 27 of 34 locations and in 10 of 23 commercial vineyards. Nine of the 23 commercial vineyards examined were *V. vinifera* (five White Riesling and four Chardonnay), 10 were *Vitis* interspecific hybrid, and four were *V. labruscana* L. H. Bailey 'Concord'. *S. titanus* was found in three White Riesling vineyards, two Chardonnay vineyards, four hybrid vineyards, and one Concord vineyard. In the Lake Erie region, near Westfield, NY, *S. titanus* was collected from wild grapevines at all four locations examined and from two of four commercial Concord vineyards. On Long Island, *S. titanus* was found on *V. riparia* at two of four locations and in two Chardonnay vineyards of the six White

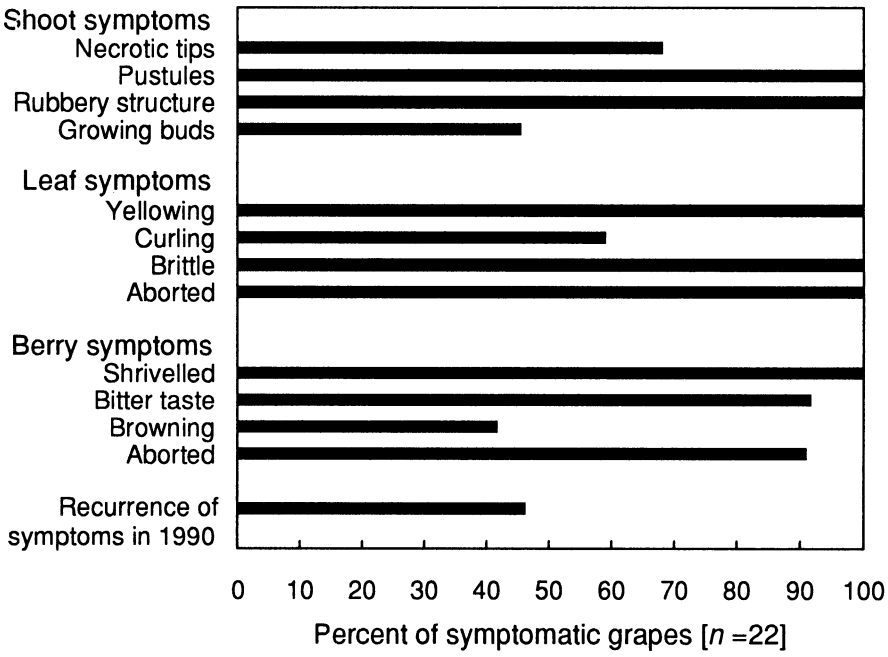

Fig. 1. Expression of symptoms of grapevine yellows in *Vitis vinifera* 'White Riesling' at Valois, NY, in 1989.

Riesling and Chardonnay vineyards examined.

The maximum density observed was 182 *S. titanus* per 100 leaves on 6 June 1990 on *V. vinifera* in Dresden, NY. All stages of leafhoppers were found on *V. riparia* and *V. vinifera,* and all stages except eggs were found on Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.). First-instar nymphs of *S. titanus* were observed on 24 May 1990, the second instar on 6 June, third instar on 12 June, fourth instar on 26 June, and fifth instar on 3 July. Adults were first observed on 9 July 1990 and 11 July 1989. Eggs of *S. titanus* were found in the bark of *V. vinifera,* and first- and second-instar nymphs were numerous in the vineyards; however, vineyard populations declined rapidly by the end of June. Most leafhoppers completed nymphal development on *V. riparia,* from which adult leafhoppers migrated into the peripheral parts of commercial vineyards.

Females of another species of *Scaphoideus* that were found occasionally could not be identified. Known vectors of peach X-disease such as *Colladonus clitellarius* (Say), *Paraphlepsius irroratus* (Say), and *Scaphytopius acutus* (Say) (21) as well as *Aphrodes bicinctus* (Schrank), a vector of clover phyllody (17), were occasionally captured by sticky traps in the vineyards.

Incidence of GY in vineyards. The incidence of GY in New York vineyards was very low. Symptoms were observed during 1989 in only one of approximately 10,000 White Riesling vines planted in 1981 at Dresden and in 22 of about 15,000 White Riesling vines and two of about 20,000 Chardonnay vines planted in 1972 at Valois. Symptoms developed shortly after bloom, beginning with aborted clusters and necrotic shoot tips. In August all symptomatic vines had rubbery shoots with pustules; yellow, brittle, or abscised leaves; and shriveled berries (Fig. 1). Symptoms such as necrotic shoot tips, growth of axillary buds, curling of the leaves, bitter berries, and aborted clusters were observed in many but not all affected vines.

With only one exception, affected vines had both symptomatic and nonsymptomatic shoots. Six (46%) of 13 examined vines that expressed symptoms at Valois in 1989 were also symptomatic in 1990, and five newly affected vines were detected in 1990. In most cases, symptomatic shoots in the second year grew from buds at the base of canes that showed symptoms in the first year. In general, these were the only buds on symptomatic shoots that survived the winter.

Only three of 10 infected dormant vines that were transplanted to the greenhouse developed symptoms such as necrotic shoot tips, yellowing and curling of the leaves, and pustules on shoots 2–3 mo after budbreak. Of these three vines, only one expressed symptoms systemically; symptoms in the others were restricted to approximately 14% and 33% of the shoots.

In Valois, diseased vines were concentrated along the border of the vineyard adjacent to a hedgerow with abundant *V. riparia.* Seventy-four percent ($n = 27$) of symptomatic White Riesling vines grew within 25 m and 93% within 50 m of the border.

Symptoms of a yellows disease were never observed on *V. riparia* growing on the borders of vineyards with symptomatic *V. vinifera* or at other locations.

Transmission experiments. Seventeen (29%) of 58 inoculated *Vicia faba* plants developed symptoms of an MLO infection within 6–10 wk (Table 1). Symptomatic beans were stunted and had small leaves with upward-curled edges. Flowers were not affected. Leafhoppers collected from *V. riparia* transmitted this disease at a higher percentage (38%) than those collected from *V. vinifera* (19%) regardless of collection site (Table 1). None of the potted Chardonnay vines fed on by *S. titanus* developed symptoms of GY in the greenhouse during the year of observation.

ELISA testing of leafhoppers. The results of a typical ELISA for detecting FD-associated antigens in field-collected leafhoppers are presented in Figure 2. The mean absorbance value of five laboratory-bred, healthy leafhoppers from Dijon, France, after 90 min was 0.292 ± 0.06, and that of 30 field-collected *S. titanus* from New York was

Table 1. Expression of symptoms of MLO infection in *Vicia faba* after feeding of field-collected *Scaphoideus titanus*

Source of leafhoppers	No. inoculated plants	Symptomatic plants		No. leafhoppers positive/negative by ELISA after feeding
		No.	%	
Valois, NY				
Vitis riparia	4	2	50	3/32
V. vinifera	10	3	30	5/51
Dresden, NY				
V. riparia	16	9	56	15/97
V. vinifera	12	2	17	12/68
Other locations				
V. riparia	12	1	8	1/12
V. vinifera	4	0	0	
Total	58	17	29	36/260
V. riparia	32	12	38	19/141
V. vinifera	26	5	19	17/119

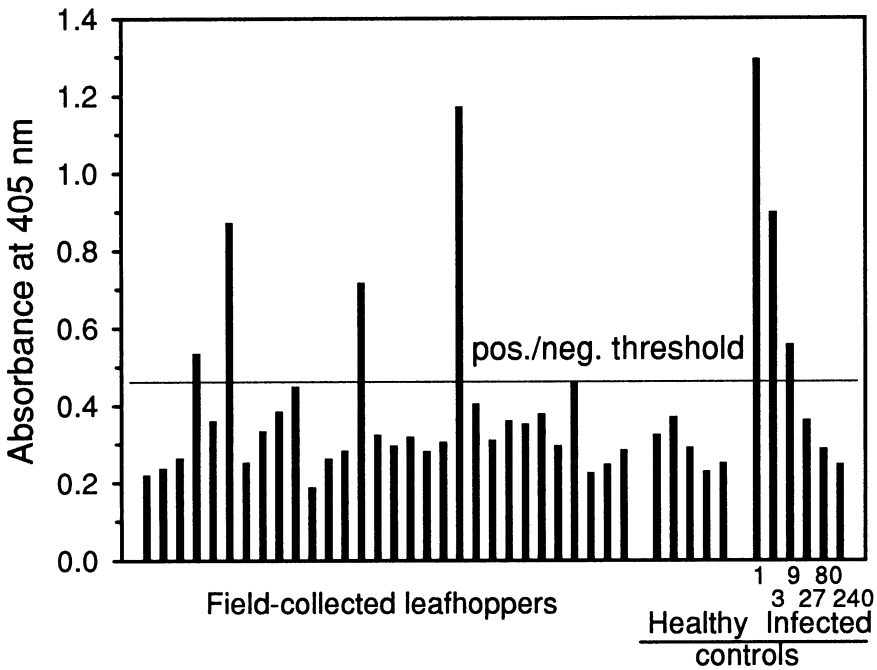

Fig. 2. Representative ELISA readings after 90 min for *flavescence dorée* (FD)-related antigens in field-collected *Scaphoideus titanus.* Bars represent absorbance at 405 nm of individual field-collected leafhoppers from New York, laboratory-bred leafhoppers (healthy controls) from France, or extracts prepared in France from several leafhoppers infected by the FD pathogen (dilutions 1:1, 1:3, 1:9, 1:27, 1:80, 1:240). Field-collected *S. titanus* were considered positive if absorbance readings at 405 nm exceeded the positive-negative threshold (mean of healthy leafhoppers plus three times the standard deviation).

0.379 ± 0.21. The readings of four field-collected leafhoppers from New York (0.535, 0.715, 0.873, 1.172), as well as of preparations of infected leafhoppers from France diluted 1:1 (1.294), 1:3 (0.898), and 1:9 (0.557), exceeded the calculated positive-negative threshold of 0.462.

Forty-eight (13%) of 371 *S. titanus* leafhoppers reacted positively (Table 2). Positive readings were recorded in 12% of the leafhoppers collected from *V. vinifera* (19/163) and in 14% collected from *V. riparia* (29/208). The percentage of positives was higher in nymphs collected from *V. riparia* (14%) than in nymphs collected from *V. vinifera* (10%), but no differences were found in adults collected from the two species. The percentage of positives was higher in male leafhoppers (20%) than in females (10%) or nymphs for which the sex could not be determined (10%) (Fig. 3). The mean absorbance readings of positive males (0.730 ± 0.07) and females (0.666 ± 0.03) were significantly ($P \geq 0.95$) higher than the mean absorbance of larvae (0.444 ± 0.09). No correlation was found between ELISA results and symptom induction in *Vicia faba* on which the tested leafhoppers had fed (Table 1).

ISEM. MLO-like structures could be observed by ISEM in all extracts obtained from field-collected, ELISA-positive leafhoppers but not in extracts from ELISA-negative laboratory-bred, healthy leafhoppers (Fig. 4). The circular structures (168 nm in diameter; $n = 75$; SD = 29) were highly decorated by antibodies to FD.

DISCUSSION

S. titanus was found to be widespread on grapevines in New York although in low numbers. In Europe, this leafhopper seems to be strictly limited to *Vitis* spp., with cultivated *V. vinifera* the most important host, whereas in New York, *V. riparia* was the preferred host. The ubiquitous wild grapevines in close proximity to vineyards are likely a refuge from which adult leafhoppers migrate to cultivated grapevines. This observation may explain the differences between FD and GY in epidemiology.

S. titanus is more abundant on *V. vinifera* in France than the densities we observed on *V. vinifera* and *V. riparia* in New York. Moutous et al (28) reported up to 800 *S. titanus* per 100 leaves in mid-June in French vineyards. This may partly explain the lower rate of disease spread in New York.

The symptoms of GY are similar to those of FD. More than 50% of the grapevines that expressed symptoms in 1989 did not show symptoms the following year, which is common for FD-infected vines, and two vines expressed symptoms systemically, which is comparable to vines newly infected by the FD agent. However, GY does not spread as rapidly as FD. In Valois the number of diseased White Riesling vines increased from two in 1983 (30) to 22 in 1989, which is similar to the rate of spread for *bois noir* (13). The expression of symptoms on shoots growing from the base of symptomatic canes from the previous year resembles symptom development observed for *Vergilbungskrankheit*.

At Valois, symptomatic vines were concentrated at the border of the vineyard, as reported by Caudwell et al (13) for *bois noir*. If *S. titanus* is the vector of GY, this distribution could be explained by the transmission of the GY pathogen from *V. riparia* at the borders of vineyards to *V. vinifera*. This is possible in spite of the fact that no symptoms of GY were observed on *V. riparia*. The absence of symptoms on *V. riparia* is not unexpected, since several American *Vitis* spp. and hybrids used as rootstocks can be infected with the FD pathogen but do not show symptoms (7,27).

Symptoms of an MLO-like disease developed on *Vicia faba* plants fed on by field-collected *S. titanus*. Similar symptoms of stunted shoots and small, upward-curling leaves are observed in *Vicia faba* infected with FD (12). We assumed that grapevines were the source of the pathogen transmitted to *Vicia faba*, because the leafhoppers used in these experiments were collected from *Vitis*. Whether the symptoms were caused by the pathogen of GY is not

Table 2. Results of ELISA testing of individual *Scaphoideus titanus* from three vineyards in New York using antibodies to *flavescence dorée*

Collection site / Development stage at collection	Leafhoppers collected from: *Vitis vinifera* No. positive/ no. tested	% positive	*V. riparia* No. positive/ no. tested	% positive	Total No. positive/ no. tested	% positive
Dresden						
Nymphs	4/43	9	11/64	17	15/107	14
Adults	7/47	15	11/65	17	18/112	16
Total	11/90	12	22/129	17	33/219	15
Valois						
Nymphs	3/29	10	3/43	7	6/72	8
Adults	5/44	11	Not tested		5/44	11
Total	8/73	11	3/43	7	11/116	9
Geneva						
Nymphs	Not tested		4/19	21	4/19	21
Adults	Not tested		0/17	0	0/17	0
Total	Not tested		4/36	11	4/36	11
Total						
Nymphs	7/72	10	18/126	14	25/198	13
Adults	12/91	13	11/82	13	23/173	13
Total	19/163	12	29/208	14	48/371	13

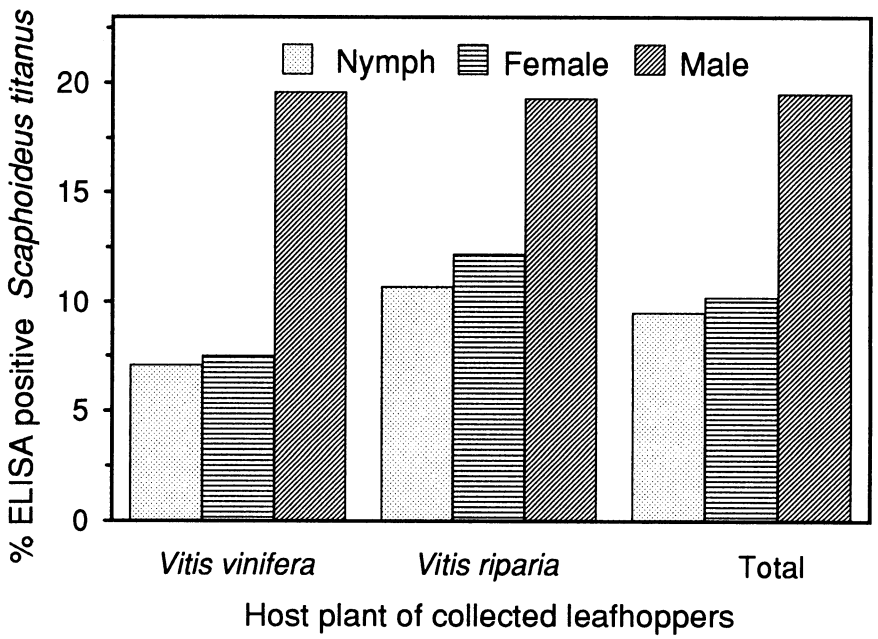

Fig. 3. Proportion of ELISA-positive *Scaphoideus titanus* nymphs ($n = 42$), males ($n = 113$), and females ($n = 216$) collected from *Vitis vinifera* in vineyards and from *V. riparia* in adjacent hedgerows.

clear, however, since none of the potted grapevines used for transmission experiments developed symptoms of GY. However, transmission of GY by *S. titanus* is perhaps more efficient to *Vicia faba* than to *V. vinifera,* or symptom expression may be delayed and less apparent in grapevine. Caudwell et al (12) reported weak symptom expression of FD-infected vines in the greenhouse, and seven of 10 symptomatic vines transplanted in 1989 from Valois to the greenhouse did not develop symptoms. Transmission of FD by *Euscelidius variegatus,* an experimental leafhopper vector, was shown to be more efficient for the herbaceous host *Vicia faba* than for *Vinca rosea* (8). Furthermore, although no symptoms of an MLO infection were found in *V. riparia,* leafhoppers collected from this species were more efficient in transmission tests than those collected from *V. vinifera.*

The positive reaction of New York leafhopper extracts with polyclonal antibodies produced to the pathogen of FD indicates that these insects contained an antigen serologically related to FD. There was no correlation between symptom induction in *Vicia faba* and a positive reaction of leafhoppers in ELISA. Usually, however, several insects died during the transmission experiments and were not used for ELISA. If one leafhopper is sufficient for transmission of the pathogen to *Vicia faba,* as it is for FD (4,8), then such a correlation cannot be expected. Boudon-Padieu et al (4) obtained positive ELISA results from FD-infected *E. variegatus* before the pathogen concentration was sufficient for transmission of the disease. One reason for the lack of correlation could be that we tested leafhoppers when the titer of the pathogen was high enough for detection but not for transmission.

The nymphal stages of *S. titanus* are not as mobile as adult leafhoppers, and they usually remain on the plants on which they hatched. Larvae reacting positively in ELISA most likely acquired the antigen from the plants on which they were collected. The positive reaction for 10% of the nymphs collected from *V. vinifera* and 14% of the nymphs collected from *V. riparia* indicates that not only cultivated grapevines in affected vineyards, but also symptomless wild grapevines in the vicinity of these vineyards were a source of this antigen. This contribution of symptom-free *V. riparia,* combined with the preference of *S. titanus* for the wild grape habitat, could explain the high proportion of ELISA-positive leafhoppers compared to the low incidence of GY in vineyards. This implies that the ubiquitous wild grapevines serve as a source of infectious leafhoppers that cause regular reinfections in vineyards, assuming that the antigen found in *S. titanus* is indeed the causal agent of GY.

The proportion of ELISA-positive males exceeded that of females. Kuszala (22) reported greater sensitivity and vector efficiency of male *E. variegatus* in reference to FD, and Boudon-Padieu et al (4) reported higher ELISA readings in males than in females. Conversely, Chiykowski and Sinha (18) reported a higher transmission efficiency of female *Macrosteles fascifrons* as vectors of the clover proliferation agent.

Considering the low incidence of GY in vineyards, the value of 13% ELISA-positive leafhoppers seems extremely high. However, the experiments suggested that most of the leafhoppers probably acquired the antigen from wild, symptomless *V. riparia.* Another explanation for the low incidence of GY could be that infections in some *V. vinifera* vineyards were latent because climatic conditions were unfavorable for replication of the GY pathogen. The duration of the latent period in vines inoculated by leafhoppers carrying the FD pathogen appears to depend on the temperature in summer (14). For example, latent periods are short in the Mediterranean region of France, where summers are long and hot, while long latent periods are common in the cooler northern viticultural areas of France.

The observation of MLOs in ELISA-positive leafhoppers is further evidence for the serological relationship of the FD pathogen and the antigens detected in *S. titanus.* Preservation of size and shape was poor because PBS was used as an extraction medium. Caudwell et al (15) obtained better results with a different medium (11) for the preservation of infectivity of MLO extracts used for leafhopper infection by injection. Nevertheless, as demonstrated by Sinha and Chiykowski (34), the method we used is suitable for reliable and specific detection of MLO infections in individual leafhoppers.

Although we cannot conclude that the MLOs found in *S. titanus* are the causal agents of GY because of the lack of experimental transmission of the pathogen to *V. vinifera* by *S. titanus* with subsequent development of symptoms, the close association of this leafhopper with grapevines in the field suggests that the same or related MLOs were acquired from *Vitis.* This study provides additional evidence for the hypothesis that *S. titanus* is the vector of GY in New York and that GY and FD are caused by related pathogens. Our identification of *V. riparia* as a host of *S. titanus,* the development of a disease with MLO-like symptoms in *Vicia faba* plants fed on by field-collected *S. titanus,* and the detection of MLOs in this leafhopper all support the hypothesis that the pathogen can be maintained through a natural cycle of transmission between *S. titanus* and wild *Vitis* spp. Nevertheless, further research is needed to confirm that this MLO is the causal agent of GY, to show that the pathogen can be detected in affected grapevines, and to demonstrate the direct transmission of GY to *Vitis* by *S. titanus.*

Even if GY and FD are closely related, FD may be caused by a more virulent and transmissible form of the pathogen, and its introduction into the United States should be avoided. No yellows disease is yet reported from California, where *S. titanus* is present (1), and care must be taken to prevent the introduction of either GY or FD into this viticultural region. Where *S. titanus* is present, the pathogen could spread rapidly through healthy vineyards, as it did in France.

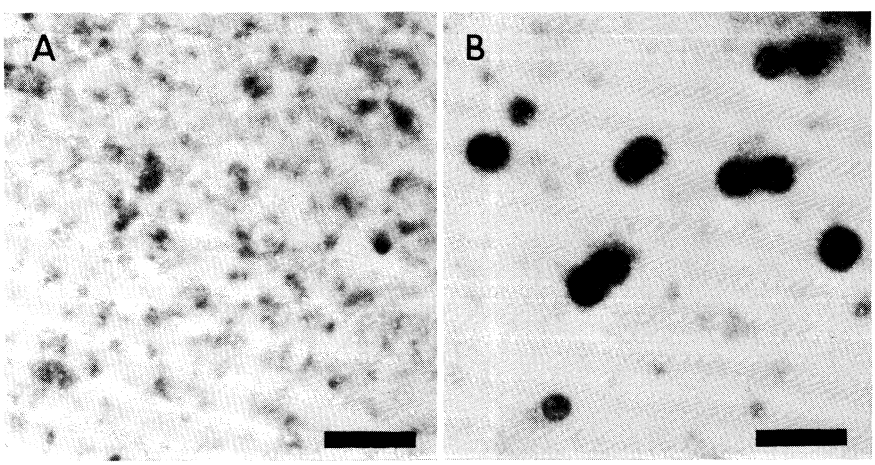

Fig. 4. Immunosorbent electron micrographs of extracts of **(A)** a laboratory-bred, healthy *Scaphoideus titanus* leafhopper and **(B)** a field-collected, ELISA-positive leafhopper (bars represent 500 nm). The immunosorbent electron microscopy used antibodies to *flavescence dorée.*

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LITERATURE CITED

1. Barnett, D. E. 1977. A revision of the Nearctic species of the genus *Scaphoideus* (Homoptera: Cicadellidae). Trans. Am. Entomol. Soc. 102:484-593.
2. Beirne, B. P. 1956. Leafhoppers (Homoptera: Cicadellidae) of Canada and Alaska. Can. Entomol. 88, Suppl. 2.
3. Belli, G., Fortusini, A., Pizzoli, L., Rui, D., and Torresin, G. 1983. Gravi danni da Flavescenza dorata in vigneti di Pinot nel Veneto. L'Inf. Agrar. 39:24431-24433.
4. Boudon-Padieu, E., Larrue, J., and Caudwell, A. 1989. ELISA and dot-blot detection of flavescence dorée-MLO in individual leafhopper vectors during latency and inoculative state. Curr. Microbiol. 19:357-364.
5. Boudon-Padieu, E., Terwisscha van Scheltinga, T., Lherminier, J., and Caudwell, A. 1987. ELISA and immunofluorescence detection of the MLO agent of the grapevine flavescence dorée on individual leafhopper vectors. Isr. J. Med. Sci. 23:506.
6. Caudwell, A. 1964. Identification d'une nouvelle maladie à virus de la vigne, "la flavescence dorée." Etude des phénomènes de localisation des symptômes et de rétablissement. Ann. Epiphyt. 15:1-193.
7. Caudwell, A. 1983. L'origine des jaunisses à mycoplasmes (MLO) des plantes et l'exemple des jaunisses de la vigne. Agronomie (Paris) 3:103-111.
8. Caudwell, A., Boudon-Padieu, E., Kuszala, C., and Larrue, J. 1987. Biologie et étiologie de la flavescence dorée. Recherches sur son diagnostic et sur les méthodes de lutte. Pages 175-208 in: Atti del Convegno sulla Flavescenza dorata della vite, Verona 1987.
9. Caudwell, A., Detienne, G., and Bachelier, J. C. 1964. Die zwei Verhaltensweisen der Flavescence dorée. Weinberg Keller 11:575-578.
10. Caudwell, A., Giannotti, J., Kuszala, C., and Larrue, J. 1971. Etude du rôle de particules de type "mycoplasme" dans l'étiologie de la flavescence dorée de la vigne. Examen cytologique des plantes malades et des cicadelles infectieuses. Ann. Phytopathol. 3:107-123.
11. Caudwell, A., and Kuszala, C. 1986. Mise au point par l'épreuve d'infectivité d'un milieu d'extraction et d'un milieu de survie, d'une méthode de purification et de conservation au froid de l'agent pathogène (MLO) de la flavescence dorée. Agronomie (Paris) 6:885-892.
12. Caudwell, A., Kuszala, C., Bachelier, J. C., and Larrue, J. 1970. Transmission de la flavescence dorée de la vigne aux plantes herbacées par l'allongement du temps d'utilisation de la cicadelle *Scaphoideus littoralis* Ball et l'étude de sa survie sur un grand nombre d'espèces végétales. Ann. Phytopathol. 2:415-428.
13. Caudwell, A., Kuszala, C., Larrue, J., and Bachelier, J. C. 1972. Responsabilité d'un vecteur aérien dans l'épidémiologie du bois noir de la vigne. Etude de l'existence de ceps sensibles et de ceps tolérants, expliquant la permanence de la maladie sur les mêmes ceps. Ann. Phytopathol. (n. hors série):171-180.
14. Caudwell, A., and Larrue, J. 1986. La flavescence dorée dans le midi de la France et dans le Bas-Rhône. Prog. Agric. Vitic. 103:517-523.
15. Caudwell, A., Meignoz, R., Kuszala, C., Schneider, C., Larrue, J., Fleury, A., and Boudon, E. 1982. Purification sérologique et visualisation au microscope électronique de l'agent pathogène (MLO) de la flavescence dorée de la vigne dans les extraits de vecteurs infectieux et dans les extraits de plantes malades. C. R. Seances Soc. Biol. 176:723-729.
16. Caudwell, A., Moutous, G., Brun, P., Larrue, J., Fos, A., Blancon, G., and Schick, J. P. 1974. Les épidémies de flavescence dorée en Armagnac et en Corse, et les nouvelles perspectives de lutte contre le vecteur par des traitements ovicides d'hiver. Minist. Agric. Bull. Tech. Inf. 294:783-794.
17. Chiykowski, L. N. 1975. *Aphrodes bicincta* as a vector of the clover phyllody agent. Ann. Entomol. Soc. Am. 68:645-648.
18. Chiykowski L. N., and Sinha, R. C. 1970. Sex and age of *Macrosteles fascifrons* in relation to the transmission of the clover proliferation causal agent. Ann. Entomol. Soc. Am. 63:1614-1617.
19. Daire, X., Schneider, B., Seemüller, E., Santoni, S., Berville, A., Boudon-Padieu, E., and Caudwell, A. 1991. DNA cloning and detection of flavescence dorée mycoplasma-like organism (MLO). Pages 484-487 in: Proc. Meet. Int. Council Study Viruses Virus Dis. Grapevine (ICVG) 10th, I. C. Rumbos, R. Bovey, D. Gonsalves, W. B. Hewitt, and G. P. Martelli, eds. 3-7 Sept. 1990, Ores Publishing, Volos, Greece.
20. Gärtel, W. 1965. Untersuchungen über das Auftreten und das Verhalten der Flavescence dorée in den Weinanbaugebieten an Mosel und Rhein. Weinberg Keller 12:347-376.
21. Gilmer, R. M., Palmiter, D. H., Schaefers, G. A., and McEwen, F. L. 1967. Leafhopper transmission of X-disease virus of stone fruits in New York. N.Y. State Agric. Exp. Stn. Bull. 813.
22. Kuszala, C. 1986. Influence du sexe et de l'âge des insectes vecteurs injectés dans l'épreuve d'infectivité des jaunisses des plantes. Mesure radiographique du volume injecté à *Euscelidius variegatus* (Kirschbaum). Agronomie (Paris) 6:591-598.
23. Lherminier, J., Prensier, G., Boudon-Padieu, E., and Caudwell, A. 1990. Immunolabeling of grapevine flavescence dorée MLO in salivary glands of *Euscelidius variegatus:* A light and electron microscopy study. J. Histochem. Cytochem. 38:79-85.
24. Lherminier, J., Terwisscha van Scheltinga, T., Boudon-Padieu, E., and Caudwell, A. 1989. Rapid immunofluorescent detection of the grapevine flavescence dorée mycoplasmalike organism in the salivary glands of the leafhopper *Euscelidius variegatus* Kbm. Phytopathol. Z. 125:353-360.
25. Magarey, P. A., and Wachtel, M. F. 1986. Australian grapevine yellows. Int. J. Trop. Plant Dis. 4:1-14.
26. Maixner, M., and Pearson, R. C. 1991. Studies on *Scaphoideus titanus* Ball, a possible vector of grapevine yellows in New York. Pages 193-201 in: Proc. Meet. Int. Council Study Viruses Virus Dis. Grapevine (ICVG) 10th, I. C. Rumbos, R. Bovey, D. Gonsalves, W. B. Hewitt, and G. P. Martelli, eds. 3-7 Sept. 1990, Ores Publishing, Volos, Greece.
27. Moutous, G., and Fos, A. 1977. Définition des symptômes de la flavescence dorée sur les variétés porte-greffes. Rev. Zool. Agric. Pathol. Veg. 76:90-98.
28. Moutous, G., Fos, A., Besson, J., Joly, E., and Biland, P. 1977. Résultats d'essais ovicides contre *Scaphoideus littoralis* Ball., cicadelle vectrice de la flavescence dorée. Rev. Zool. Agric. Pathol. Veg. 76:37-49.
29. Munoz, A. H., and Rodriguez, G. M. 1972. La flavescence dorée en el valle de Elqui. Bol. DAV 3:7-19.
30. 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.
31. Rumbos, I. 1979. Studies on the etiology of a yellows disease of grapevines in Greece. J. Plant Dis. Prot. 86:266-273.
32. Schvester, D., Carle, P., and Moutous, G. 1961. Sur la transmission de la flavescence dorée des vignes par une cicadelle. C. R. Seances Acad. Agric. Fr. 47:1021-1024.
33. Schwartz, Y., Boudon-Padieu, E., Grange, J., Meignoz, R., and Caudwell, A. 1989. Obtention d'anticorps monoclonaux spécifiques de l'agent pathogène de type mycoplasme (MLO) de la flavescence dorée de la vigne. Res. Microbiol. 140:311-324.
34. Sinha, R. C., and Chiykowski, L. N. 1986. Detection of mycoplasmalike organisms in leafhopper vectors of aster yellows and peach X-disease by immunosorbent electron microscopy. Can. J. Plant Pathol. 8:387-393.
35. Sutula, C. L., Gillett, J. M., Morrissey, S. M., and Ramsdell, D. C. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70:722-726.