Fieberiella florii (Homoptera: Auchenorrhyncha) as a Vector of “*Candidatus Phytoplasma mali*”

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ABSTRACT

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Laboratory trials were carried out to transmit “*Candidatus Phytoplasma mali*” to healthy apple seedlings with the leafhopper *Fieberiella florii*. Experiments on serial inoculation access period and molecular analyses performed on test plants and insects confirmed the ability of the leafhopper to carry and transmit the phytoplasma. Field surveys by means of yellow sticky traps were conducted in northwestern Italy to verify the abundance and the natural infectivity of *F. florii* in apple orchards and in wild vegetation in areas surrounding apple orchards. Despite the high percentages of infected specimens obtained in the apple orchards (5.7%) and in the wild vegetation areas (20.0%), the risk of apple tree infection by *F. florii* in nature is probably low because of the very low insect density recorded. In spite of the low number of specimens collected, the presence of the leafhopper in apple orchards in summer, when the main vector, the psyllid *Cacopsylla melanoneura*, feeds on alternative hosts, is meaningful. Moreover, the high degree of polyphagy of the leafhopper opens up new interesting prospects for the epidemiology of apple proliferation.

Additional keywords: apple proliferation, Cicadellidae, population dynamics

The apple proliferation (AP) phytoplasma represents one of the most economically important threats to apple trees in central and southern Europe. Recently, the novel designation of “*Candidatus Phytoplasma mali*” was proposed to describe this phytoplasma (25); and this taxonomic designation will be followed in the present work.

The psyllids *Cacopsylla melanoneura* (Förster) and *C. picta* (Förster) (synonym *C. costalis* (Flor)) are acknowledged as the main vectors of “*Ca. Phytoplasma mali*” in northern Italy and Germany (6,10,11, 29,30). These insects colonize apple orchards in winter and spring, before migrating to alternative hosts (6,30). In spite of this short time on apple trees and the relatively low infection of the insects, disease incidence is increasing, probably because of the high population density observed, especially for overwintered adults (19,31). Nevertheless, the question has not yet been solved whether other vector species, apart from psyllids, can be involved in the natural spread of “*Ca. Phytoplasma mali*”, especially during summer months until the leaves fall.

The leafhopper *Fieberiella florii* (Stål), recognized in North America as one of the most important vectors of X-disease (8,32), was regarded, at the end of the 1980s, as a potential vector of fruit tree phytoplasmas belonging to the AP (16Sr-X) group on the basis of symptom expression and fluorescence microscopy (12) after transmission trials to healthy apple plants. Those results never have been confirmed, and there were no means to confirm the identity of the transmitted phytoplasma when the experiments were done originally. Since there is no alternative vector of “*Ca. Phytoplasma mali*” in our regions that is hosted by apple trees and can transmit that phytoplasma (i.e., *C. picta*), new investigations on the subject were required.

The objective of the present work was to determine if *F. florii* could transmit the causal agent of AP to apple. The presence of this insect in apple orchards in the Aosta Valley was determined in relation to the other leafhopper fauna in order to verify its real role as an alternative vector of “*Ca. Phytoplasma mali*” by means of surveys on population dynamics and natural infectivity, both in apple orchards and in wild vegetation areas.

MATERIALS AND METHODS

The research was organized with transmission trials carried out in 1999 to ascertain the ability of *F. florii* to transmit “*Ca. Phytoplasma mali*” to healthy apple plants. Then 4 years (2000 to 2003) of field sur-

veys were carried out to verify the population dynamics of *F. florii*, supported also by molecular analysis to check the natural infectivity of this leafhopper with “*Ca. Phytoplasma mali*”.

Insect rearing. Mass rearing of *F. florii* was carried out using nymphs and adults collected in the field from *Ligustrum vulgare* L. in Piedmont, northwestern Italy. The plants of *L. vulgare* were chosen in fields located very far from apple orchards to avoid possible phytoplasma contaminations. The leafhoppers were caged in Plexiglas containers (200 × 200 × 300 mm), with two sides and the top made of a fine gauze, on a European privet plant and reared for at least five generations before using them in transmission trials. The mass rearing cages were maintained outdoors throughout the year. Samples of 20 *F. florii* adults, as well as samples of the European privet plants used in the rearing, were checked annually by polymerase chain reaction (PCR) to ascertain the absence of phytoplasmas.

Source of inoculum. Potted apple plants grown from seed and previously inoculated with *C. melanoneura* overwintering adults collected in apple orchards located in northwestern Italy and showing high levels of AP infection were used as the source of inoculum. Before the inoculated apples were used in the transmission trials, the presence of “*Ca. Phytoplasma mali*” in the plants was confirmed by PCR.

Test plants. Healthy potted apple plants (6 to 12 months old) started from seed and grown under leafhopper-proof screenhouses (20/10 mesh) were used as test plants during the transmission trials. At the end of the experiments, test plants were treated with insecticides (0.08% permethrin, 0.15% bromopropilate) (Reckitt & Colman, Milano, Italy), then they were maintained inside a leafhopper-proof screenhouse (20/10 mesh) and regularly checked for symptom expression. At the end of the summer, when the titer of phytoplasmas is higher in the aerial part of apple trees (24), the plants were tested for the presence of “*Ca. Phytoplasma mali*” by PCR. Due to the low titer and the uneven distribution of phytoplasmas commonly observed in woody plants, the presence of AP-phytoplasmas was checked for two successive years, to avoid false negatives.

Transmission trials. In 1999, 250 third and fourth instar nymphs of *F. florii* were collected from the mass rearing to obtain a

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sufficient number of positive insects for the transmission trials. They were isolated on AP-infected apple plants for an acquisition-access period (AAP) of 10 days and then on seedlings of healthy apple plants for an incubation period of 20 days. Fifty *F. florum* died before the completion of the incubation period, and 140 were used in the transmission trials. The leafhoppers were caged in batches of 10 specimens on test plants for an inoculation-access period (IAP) of at least 10 days. When possible (due to the survival of the insects), three serial IAPs were performed for each group of insects. The *F. florum* specimens that comprised one group were kept together as a cohort throughout the experiment.

At the end of the trials, samples of the surviving insects were collected and analyzed by PCR singly to detect “*Ca. Phytoplasma mali*”.

The probability of AP transmission for a single leafhopper during each IAP was estimated by its maximum-likelihood estimator, $\hat{p}$, calculated according to Swallow (27): $\hat{p} = 1 - H^{1/k}$, where H is the observed fraction of healthy or noninfected plants and k is the number of insect vectors put on the same test plant, 10 in the case of the first and the second IAP. Considering the mortality of the insects during the trials, a mean number of eight leafhoppers per cage was calculated for the third IAP. This estimate was used also to compare the results obtained in the present paper with those concerning the ability of the psyllid *C. melanoneura* to transmit AP (29).

Field surveys. To identify the Auchenorrhyncha fauna present in the apple orchards, field surveys with yellow sticky traps (145 × 205 mm; Rebell Amarillo, Forschungsanstalt Wädenswil, Switzerland) were carried out in 2000 in the Aosta Valley (northwestern Italy), a region with an important tradition of apple production and where symptoms ascribable to infection by “*Ca. Phytoplasma mali*” were recorded commonly in the last 10 years.

Three apple orchards, characterized by trees of different ages and different growing systems, were chosen near the city of Aosta, at an altitude between 580 and 720 m. Three yellow sticky traps per orchard were placed on a diagonal line and maintained from the end of May to the end of September. The traps were changed weekly, and the number of Auchenorrhyncha, normally known as phytoplasma vectors, was recorded in the laboratory. In the orchards under investigation, three insecticide treatments were applied per year: mineral oil during the winter, pirimicarb (Solplant, Milano, Italy) in May, and chlorpyrifos-methyl (DowElanco, Milano, Italy) at the end of July.

To better assess the population dynamics of *F. florum*, trap samplings were repeated in the years 2001 to 2003 in the same apple orchards and with the same modalities, except for 2003 when the sam-

plings lasted until the end of October. In 2003, new samplings were carried out in three other areas (C1, C2, C3) around apple orchards in wild vegetation, mainly *Crataegus* spp. and a few *Rubus* spp. and *Ligustrum* spp., to evaluate the spread of *F. florum*, widely acknowledged as a highly polyphagous insect (28), in the neighborhood of apple orchards. In this case, the traps were smaller (80 × 150 mm; Rebell Giallo, Forschungsanstalt Wädenswil, Switzerland) because of the restricted size of the wild areas, and they were changed weekly. The number of *F. florum* was recorded in the laboratory, and all the specimens collected in 2003 were removed from sticky traps using a drop of xylene, washed in xylene to remove the glue, and dried before DNA extraction and molecular analysis to verify the natural infection of the insects by “*Ca. Phytoplasma mali*”.

DNA extraction and molecular analysis. Plant DNA was extracted from 1 g of leaf midribs from each test plant and source of inoculum. A phytoplasma enrichment procedure described by Ahrens and Seemüller (1) was followed as reported in Tedeschi et al. (30). Total insect DNA was extracted from each leafhopper following a procedure previously developed by Marzachi et al. (16).

To ascertain the absence of any phytoplasma in *F. florum* mass rearing, the leafhopper and the plant DNA were analyzed by means of the universal primer pairs P1/P7 (23), F2/R2 (14), and P1/Tint (26). Moreover, the amplicons obtained with PCR performed using P1/P7 were nested, after 1/40 dilution, with the primer pair fO1/rO1 (15), specific for the AP group to reveal also possible low titers of those phytoplasmas. Reaction and cycling conditions were as described in the original papers for the respective primer sets.

The presence of “*Ca. Phytoplasma mali*” was determined from test plants and insects involved in the transmission trials as well as from specimens collected on yellow sticky traps by PCR assays. First the generic primers P1/Tint and then the AP-specific group primer fO1/rO1 in nested PCR, after a direct PCR performed using the phytoplasma universal primer pair P1/P7, were used. Amplification products were analyzed in 1% agarose gels and stained with ethidium bromide.

Amplicons obtained with fO1/rO1 primers were further digested with the restriction endonuclease *SspI* (Sigma-Aldrich Srl, St. Louis, MO, USA), to confirm the presence of the “*Ca. Phytoplasma mali*” by restriction fragment length polymorphism (RFLP) analyses (14). Seven microliters of the amplicon was digested with 3 U of *SspI* for 4.5 h at 37°C. The specimens collected on yellow sticky traps were also digested with the restriction endonuclease *RsaI* (New England BioLabs, Beverly, MA, USA). Seven microliters of the amplicon was digested with 5 U of *RsaI* at 37°C overnight. Five leaf samples collected from healthy apple plants obtained from seed, as well as five samples of *F. florum* adults collected from the mass rearing, were used as controls.

RESULTS

PCR-RFLP detection. None of the *F. florum* and plant samples collected from the mass rearing gave amplification products after direct PCR with the universal primer pairs P1/P7, F2/R2, and P1/Tint as well as after nested PCR with the primer pair fO1/rO1 (data not shown). Amplification products of the expected size were obtained from all apple plants used as inoculum sources, some of the experimentally infected insects and test plants, and some

Fig. 1. Polymerase chain reaction (PCR) amplification with the generic primer pair P1/Tint of total DNA from experimentally infected *Fiebertiella florum* adults (1 and 2); insect-inoculated apple seedlings (3 and 4); *F. florum* adults collected in the apple orchards (5 and 6); *F. florum* adults collected in the wild vegetation areas (7 and 8); apple proliferation (AP) control (9); healthy *F. florum* adult (10); healthy apple seedling (11); water control (12); 1-kb DNA ladder (Invitrogen, Carlsbad, CA) (13).

F. florum adults collected in the field, after direct PCR using the P1/Tint primer pair and nested PCR using the fO1/rO1 primer pair that is specific for the AP-group phytoplasma. There was good correspondence between the amplicons obtained with P1/Tint and fO1/rO1. The restriction profiles of the fragments amplified with the specific primers fO1/rO1 in nested PCR confirmed the presence of “*Ca. Phytoplasma mali*” with either of the restriction endonucleases used (Figs. 1 to 3).

Transmission trials. Two groups of leafhoppers were able to complete only the first IAP, because most of the insects died before the end of the trial. Six groups also were able to complete the second IAP, while six batches completed all three IAPs, so a total number of 32 apple seedlings were inoculated with *F. florum* that had experimentally acquired “*Ca. Phytoplasma mali*”. The insects used in the first IAP were mostly fifth instar nymphs, whereas the specimens used in the second and third IAPs were adults.

One of 14 (7.1%) plants inoculated during the first IAP tested positive for AP phytoplasma after nested PCR, while during the second and third IAPs, 4 of 12 (33.3%) and 1 of 6 (16.7%) test plants, respectively, yielded amplicons in nested assays using fO1/rO1 primers (Table 1). Infected test plants gave positive PCR results only the year after inoculation via insect. All the plants that acquired the phytoplasma showed typical symptoms of apple proliferation disease. The develop-

Fig. 2. 1-9, Polymerase chain reaction (PCR) amplification with the apple proliferation (AP)-group specific primer pair fO1/rO1 in nested PCR after a direct PCR with the primer pair P1/P7 of total DNA from: experimentally infected *Fiebertella florum* adults (1 and 2); insect-inoculated apple seedlings (3 and 4); AP control (5); healthy *F. florum* adult (6); healthy apple seedling (7); water control (8); 1-kb DNA ladder (Invitrogen, Carlsbad, CA) (9). 10-18, Restriction fragment length polymorphism (RFLP) analysis with *SspI* restriction enzyme of fO1/rO1 amplicons from: experimentally infected *F. florum* adults (10 and 11); insect-inoculated apple seedlings (12 and 13); AP control (14); pear decline (PD) control (15); European stone fruit yellows (ESFY) control (16); undigested AP control (17); 100-bp ladder (Invitrogen) (18).

Fig. 3. 1-8, Polymerase chain reaction (PCR) amplification with the apple proliferation (AP)-group specific primer pair fO1/rO1 in nested PCR after a direct PCR with the primer pair P1/P7 of total DNA from: *Fiebertella florum* adults collected in the apple orchards (1 and 2); *F. florum* adults collected in the wild vegetation areas (3 and 4); AP control (5); healthy *F. florum* adult (6); water control (7); 1-kb DNA ladder (Invitrogen, Carlsbad, CA) (8). 9-23, Restriction fragment length polymorphism (RFLP) analysis with *SspI* and *RsaI* restriction enzyme of fO1/rO1 amplicons from: *F. florum* adults collected in the apple orchards (9, 10, 17, and 18); *F. florum* adults collected in the wild vegetation areas (11, 12, 19, and 20); AP control (13 and 21); pear decline (PD) control (14 and 22); European stone fruit yellows (ESFY) control (15 and 23); 100-bp ladder (Invitrogen) (16 and 24).

ment of long stipules and the production of basal suckers and leaf reddening could be observed at the end of the summer following inoculation, but symptoms were more evident in the following year. Production of early shoots was often noticed in the spring of the following year. The probability of AP transmission by a single *F. florii* assessed by its maximum-likelihood estimator was 0.007, 0.040, and 0.022 during the first, second, and third IAP, respectively. Statistical analysis of the results of molecular diagnoses on test plants revealed the lack of significant differences among the three IAPs (Fisher's exact test, $P > 0.05$).

Three of 80 (3.8%) *F. florii* analyzed at the end of the trials were positive for the AP phytoplasma after nested PCR with the primer pair fO1/rO1. Two of those adults were members of the two groups that transmitted AP only during the second IAP. The third positive *F. florii* belonged to the

group that transmitted AP during all three IAPs.

Field surveys. In 2000, different Auchenorrhyncha were captured by the yellow sticky traps. The most abundant species were *F. florii* with an average of 4.7 ± 5.0 ($\pm$ SD) specimens per orchard and *Anoplotettix fuscovenosus* (Ferrari) with an average of 2.7 ± 1.5 specimens per orchard. Other species, such as *Reptalus* spp., *Philaenus spumarius* (L.), and *Allygus* spp., were collected occasionally (1.7 ± 2.9 , 1.7 ± 0.6 , 1.7 ± 2.1 specimens per orchard, respectively).

In the 3 years (2001 to 2003), the number of *F. florii* collected in the three apple orchards using yellow sticky traps was always very low. A peak of only 3, 7, and 9 specimens per orchard per week was reached in 2001, 2002, and 2003, respectively (Fig. 4). The peak occurred around the second half of September and the be-

ginning of October. The presence of *F. florii* in the apple orchards in the other periods was always sporadic.

The field surveys with yellow sticky traps placed in the wild vegetation areas allowed collection of *F. florii* in all three areas. The peak was reached in the second and third weeks of September in the areas C1 and C2, and in the first week of October in the area C3. The population of the leafhopper never exceeded five specimens per area per week (Fig. 5).

Considering all the insects collected in the three apple orchards, only 3 of 58 were positive for "*Ca. Phytoplasma mali*". Higher infection percentages were recorded in *F. florii* captured in the wild vegetation areas, reaching 20% in site C2 (Table 2).

DISCUSSION

The present work gave molecular evidence that *F. florii* is a vector of the AP

Table 1. Serial inoculation access periods (IAP) of apple proliferation phytoplasma to potted apple seedlings using experimentally infected *Fiebertiella florii*

Group	IAP								
	I			II			III		
	<i>F. florii</i>	Test plant		<i>F. florii</i>	Test plant		<i>F. florii</i>	Test plant	
	N	PCR+/tested	PCR	N	PCR+/tested	PCR	N	PCR+/tested	PCR
1	10	0/3	-	nt ^a	nt	nt	nt	nt	nt
2	10	0/3	-	nt	nt	nt	nt	nt	nt
3	10	0/0	-	10	0/3	-	nt	nt	nt
4	10	0/0	-	10	0/3	+	nt	nt	nt
5	10	0/0	-	10	0/6	-	nt	nt	nt
6	10	0/0	-	10	0/6	-	nt	nt	nt
7	10	0/0	-	10	0/7	-	nt	nt	nt
8	10	0/0	-	10	1/7	+	nt	nt	nt
9	10	0/0	-	10	0/0	-	9	0/8	-
10	10	0/0	-	10	0/0	-	10	0/9	-
11	10	0/0	-	10	0/0	+	9	1/9	-
12	10	0/0	-	8	0/0	-	5	0/4	-
13	10	0/0	-	10	0/0	-	8	0/6	-
14	10	0/0	+	7	0/0	+	6	1/6	+

^a nt = not tested, insect died.

Fig. 4. Seasonal abundance of adults of *Fiebertiella florii* in Aosta Valley (northwestern Italy) apple orchards in 2000 to 2002 determined as mean number of specimens captured by all yellow sticky traps per orchard per week.

phytoplasma, confirming the results based on symptom expression and fluorescence microscopy reported by Krczal et al. (12). PCR and RFLP allowed the specific detection of “*Ca. Phytoplasma mali*” not only in the body of experimentally infected leafhoppers, but also in leaf samples of young apple plants experimentally inoculated with *F. florum* specimens, confirming the vector ability of this species.

The primer pair P1/Tint allows detection of a phytoplasma infection, in insects and plants, in direct PCR. But to determine the group to which these organisms belong, a nested PCR with group-specific primers is required, that is, fO1/rO1 in the case of AP-group phytoplasmas. Moreover, RFLP analysis enables the specific detection of “*Ca. Phytoplasma mali*”.

In the experiments performed with *F. florum*, normally the AP symptoms were more obvious in the year after the inoculation, while in the trials carried out with *C. melanoneura* (29), long stipules, witches’ brooms, and production of basal suckers were easily observed during the summer following inoculation. This gap in symptom development, combined with a belated PCR response, may be due to the later inoculation with *F. florum* (end of spring-summer), than with *C. melanoneura* (end of winter-spring), which resulted in a different physiological period of inoculation and entailed a later internal distribution of the phytoplasma in the plants.

A good correspondence between PCR-positive plants and infected insects has been observed. When a test plant was found to be infected by the “*Ca. Phytoplasma mali*” by means of molecular diagnosis, at least one of the leafhoppers caged on that plant was positive for the “*Ca. Phytoplasma mali*” using nested PCR. In only one case was there a lack of correspondence between the infectivity of the

test plant and insects, but only three samples of *F. florum* were tested, and probably the plant was infected by an insect that died during the second IAP and was not tested.

The statistical analysis did not reveal a significant difference between the proportions of positive test plants observed during the three IAPs, although the percentage of infected plants after the second IAP seems to be higher compared with the others. Likewise, the probability of AP transmission for a single leafhopper estimated by its maximum-likelihood estimator seems to be higher in the second IAP compared with the other two IAPs. This can be explained with the typical transmission patterns of several circulative, propagative mollicute plant pathogens. After a latent period of at least 12 days, the transmission rates increase progressively to a maximum and then decline (5,20).

The estimated probability of “*Ca. Phytoplasma mali*” transmission by a single insect can be a tool to compare transmission efficiency between *F. florum* and the psyllid *C. melanoneura*, acknowledged to be the main vector of AP in northwestern Italy (29,30). A comparison between the two species is worthwhile, but not easy.

First of all, due to the fact that *C. melanoneura* cannot complete its entire life cycle in the laboratory under completely controlled conditions, experimental

transmission trials with a controlled acquisition period could be carried out only with nymphs or newly emerged adults (29). But the newly emerged adults cannot survive on apple trees for a long time; consequently a relatively low transmission efficiency occurs. If we estimate the probability of transmission by a single *C. melanoneura* using the results obtained by Tedeschi and Alma (29), a percentage of 0.7 can be calculated with springtime adults experimentally inoculated. In contrast, naturally infected, overwintered adults yielded higher proportions of transmission that varied in relation to the infection rate of the source plants, i.e., 8.4% in the case of psyllids coming from an apple orchard located in Piedmont with 85% infection and 1.4% in the case of psyllids coming from apple orchards located in the Aosta Valley with different rates of infection. If we compare the trials carried out with the psyllids coming from the 85% AP-infected orchard, where most of the psyllid had the opportunity to feed on a diseased plant, to trials performed with a controlled acquisition period, we can estimate a higher transmission efficiency of *C. melanoneura* (8.4%) compared with *F. florum* (0.7, 4.0, and 2.2% in the three IAPs, respectively).

The field surveys using yellow sticky traps carried out in 2000 provided an assessment of the Auchenorrhyncha fauna

Table 2. Nested polymerase chain reaction (PCR) detection using primer pair fO1/rO1 of “*Candidatus Phytoplasma mali*” in *Fieberiella florum* specimens captured on yellow sticky traps placed in apple orchards and wild vegetation areas located in the Aosta Valley, northwestern Italy

	Apple orchards			Wild vegetation areas		
	1	2	3	C1	C2	C3
N. AP-infected ^a /tested by PCR (%)	0/1 (0)	3/53 (5.7)	0/4 (0)	1/11 (9.1)	3/15 (20.0)	2/12 (16.7)

^a Number apple proliferation-infected.

Fig. 5. Seasonal abundance of adults of *Fieberiella florum* in Aosta Valley (northwestern Italy) wild vegetation areas (C1, C2, C3) in 2003 determined as total number of specimens captured by yellow sticky traps per area per week.

present in the apple orchards in late spring-summer, a period in which the psyllids are not present on apple trees. Apart from *F. florii*, the presence of *Ph. spumarius* is noteworthy. This species was described as being able to transmit apple proliferation from infected celery to apple seedlings and from infected to healthy celery (9), but this has never been confirmed. Based on the results obtained by the transmission trials and since the presence of *F. florii* has been confirmed in apple orchards, our research focused only on this species. The samplings conducted in the period 2001 to 2003 revealed that the population of *F. florii* in the apple orchards was not high, and this was one of the greatest differences compared with the population density recorded for *C. melanoneura* (30). Moreover, it is obvious that the distribution in apple orchards is not uniform, possibly due to the wild vegetation surrounding each orchard. So, even if the percentage of AP-infected leafhoppers is much higher, especially in the wild vegetation areas (up to 20%), compared with the estimated proportion of AP-infected *C. melanoneura* (lower than 4%) (31), the relative risk of apple trees being infected by *F. florii* is probably low because of its low density and relatively inefficient transmission capability. However, the presence of *F. florii* is relevant because it colonizes the apple orchards during a period in which the psyllids, the predominant vectors of AP, are on alternative hosts (6,30) and when the titer of phytoplasmas in apple trees is higher (22). In fact, the population peaks observed for *F. florii* are in the second half of September and the beginning of October.

In America, several leafhoppers such as *Scaphytopius acutus* (Say), *Paraphlepsius irroratus* (Say), *Colladonus montanus* (Van Duzee), *C. clitellarius* (Say), *F. florii*, *Gyponana lamina* DeLong, and *Norvellina seminuda* (Say) are known vectors of peach X-disease (7,17,21). This phytoplasma contributes to the major economic loss of peach and cherry in North America and belongs to the X-disease group (16SrIII) (13). In contrast, in Europe, phytoplasmas causing fruit tree diseases belong to the apple proliferation group (16SrX) (13) and are normally associated with psyllids as vectors (2–4,6,10,11,18,29,30).

The confirmation of *F. florii* as a vector of “*Ca. Phytoplasma mali*” is also interesting for two reasons. First, this result highlights the possibility that this leafhopper harbors and hence transmits two phylogenetically distant groups of phytoplasmas (X-disease and apple proliferation), opening new perspectives in the study of phytoplasma-vector specificity. The association with one phytoplasma or the other seems to be restricted to a specific geographical region where the phytoplasmas are present. Moreover, the possibility of a leafhopper, instead of a psyllid, vectoring

the apple proliferation phytoplasma revealed new, interesting aspects on the epidemiology of the disease, in particular due to the life history of the insects.

This means that the role of *F. florii* in the transmission of apple proliferation should not be underestimated, because it is polyphagous. It appears that the range of plant species that can be infected by a given phytoplasma in nature is determined largely by the number of insect vector species that are capable of transmitting the phytoplasma and by the feeding behaviors (monophagy, oligophagy, and polyphagy) of these vectors (13). The ability of *F. florii* to colonize and feed on many plant species, mainly Rosaceae (28), suggests additional future research. In particular, further studies are needed to understand the ability of plant hosts other than apple trees to harbor “*Ca. Phytoplasma mali*” and thus to act as a source, not only of the vector, but also of the phytoplasma.

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