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Gone-wild grapevines in forests may act as a potential habitat for 'Flavescence dorée' phytoplasma vectors and inoculum

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Abstract

'Flavescence dorée' (FD) is a quarantine grapevine disease associated with FD phytoplasmas (FDp). In Switzerland, FD was first reported in 2004 in the southern part of the Canton Ticino, from where, within a few years, it spread to nearly the entire vineyard area of the Southern Alps, despite the mandatory control measures. The aim of this study was to assess the possible role of gone-wild grapevines (GWGVs) in forests resulting from early abandoned vineyards as a habitat for the main FDp vector, *Scaphoideus titanus*, and for the best alternative vector candidate, *Orientus ishidae*, as well as an FDp inoculum reservoir in Southern Switzerland. Leaf samples were collected in 20 plots and tested for the presence of FDp. Moreover, fifteen of these plots were monitored with yellow sticky traps to determine the presence and infection status of insect vectors. Finally, a hatching experiment under controlled conditions was conducted to investigate the possible oviposition activity by FDp vectors, using wood collected from GWGVs present in 11 forest sites in the surroundings of cultivated vineyards. GWGVs in forests were confirmed to act as an FDp reservoir and to represent a suitable habitat for FDp vectors (in particular, for *S. titanus*). Abandoned vineyards should be included in the FD management strategy and their systematic rogueing should be applied as soon as possible and in particular before the transition from vineyard to forest occurs, in order to avoid the survival of *S. titanus* and the establishment of inoculum in uncontrolled landscape compartments.

KEYWORDS
egg development, grapevine yellows, insect vectors, *Orientus ishidae*, *Scaphoideus titanus*, *Vitis* spp

1 | INTRODUCTION

'Flavescence dorée' (FD) is a quarantine grapevine disease associated with FD phytoplasmas (FDp, '*Candidatus* Phytoplasma vitis'), which belong to the ribosomal subgroups 16SrV-C and D (Caudwell, 1957; Davis & Dally, 2001; Lee et al., 2004). Following the first reports of FD in the 1950s in south-western France (Caudwell, 1957), the Nearctic leafhopper *Scaphoideus titanus* (Ball, 1932) was identified as its main vector, responsible for the rapid spread of the disease within vineyards (Chuche & Thiéry, 2014; Schvester et al., 1961). In Europe, *S. titanus* sustains its entire life cycle exclusively on *Vitis* species and is considered to be oligophagous (Chuche & Thiéry, 2014). Papura et al. (2012) showed a genetic homogeneity of European populations of *S. titanus*, suggesting a single introduction from North America

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later followed by spread due to human activities. In Switzerland, *S. titanus* was first reported in the Canton Ticino in 1967 (Baggiolini et al., 1968), in the Canton of Geneva in 1996 and in the Cantons of Vaud and Valais in 2007 and 2016, respectively (Linder et al., 2019).

Currently, FD is present in many European winegrowing regions despite the mandatory control measures, which consist of the application of insecticides against the main FDp vector *S. titanus*, the removal of FDp-infected grapevines, and the use of certified propagation material only (Jeger et al., 2016; Tramontini et al., 2020). In Switzerland, FD was first reported in 2004 in the southern part of the Canton Ticino even though *S. titanus* was present as early as the end of the 1960s (Baggiolini et al., 1968; Schaerer et al., 2007). Despite the immediate and systematic application of the mandatory control measures, FD spread to nearly the entire vineyard area of the Southern Alps within only a few years (Jermini et al., 2014). More recently, FD hotspots have also been identified in the eastern part of the country, namely, in the Cantons of Vaud, Geneva and Valais (Canton of Valais, 2022; Schaerer et al., 2007). The lack of success of the mandatory containment and eradication measures and the related spread of the FD epidemics have been mainly attributed to (i) the use of uncertified propagation material which was not treated with hot water, which may cause the introduction of FDp-infected grapevines into previously FDp-free locations (Caudwell, 1990; Caudwell et al., 1997), (ii) the inability to ensure the systematic eradication of FDp-infected grapevines (Jeger et al., 2016), (iii) the possible role of additional epidemiological cycles induced by alternative FDp vectors and host plant species (Jarausch et al., 2021; Malembic-Maher et al., 2020; Rizzoli et al., 2021) and (iv) the role of abandoned vineyards where *S. titanus* populations are not controlled and FDp-infected grapevines are not eradicated (Lessio et al., 2007; Ripamonti et al., 2020). These last two points may be particularly relevant for the traditional vineyard agroecosystem of Southern Switzerland, which consists of small to midsize plots embedded in areas of differing land use, including the forest area. Regarding the latter, Wyler et al. (2021) calculated that approximately 43.1% (i.e., 440 km) of the overall vineyard perimeters are less than 25 m away from the nearest forest edge. This situation may represent a higher phytosanitary risk for the vineyards due to the proximity of woody species such as *Alnus glutinosa* and *Corylus avellana*, which are the most important host plants for the best alternative FDp vector candidate, *Orientus ishidae* (Matsumura, 1902) and, especially in the case of *A. glutinosa*, an FDp inoculum reservoir in the landscape compartment of Southern Switzerland (Casati et al., 2017; Mehle et al., 2019; Rizzoli et al., 2021). Furthermore, a minor role by putative vectors such as the Nearctic leafhoppers *Japananus hyalinus* (Osborn, 1900), *Graphocephala fennahi* (Young, 1977) and *Hishimonus hamatus* (Kuoh, 1976) cannot be excluded, as these species were already found harbouring FDp genotypes compatible with *S. titanus* and grapevine in Southern Switzerland (Belgeri et al., 2021). The role of other FDp epidemiological cycles described in the literature, such as those involving the vector *Dictyophara europaea* (Linnaeus, 1767) in association with *Clematis vitalba* (Filippin et al., 2009), or *Allygus* spp. with *A. glutinosa* (Malembic-Maher et al., 2020) seems to be negligeable in the Swiss Southern Alps because of the rather low occurrence and abundance of the mentioned vectors (Trivellone et al., 2016).

The proximity of vineyard plots to the forest compartment not only implies a higher phytosanitary risk (Adrakey et al., 2022) but also represents one of the main drivers of vineyard abandonment by the winegrowers due to the related additional workload (Wyler et al., 2022). In this sense, the abandonment of the marginal winegrowing areas in Southern Switzerland is a deep-rooted and long-lasting phenomenon that has accelerated during the last century due firstly to the phylloxera epidemic caused by *Daktulosphaira vitifoliae* (Fitch, 1855) and secondly to the socio-economic evolution over the prevailing post-war period (Krebs & Bertogliati, 2017). More recently, the additional challenges posed by the ongoing climate change represent further driving factors leading to a progressive abandonment of viticulture in Southern Switzerland, in particular for vineyards managed by small-scale and part-time vignerons (Bardsley et al., 2023). Regarding the handling of abandoned vineyards, winegrowers are legally obligated to re-establish or eradicate them, a measure introduced largely for phytosanitary reasons (Fedlex, 1998, 2018). Nevertheless, especially in the past, grapevines may not have been properly eradicated and vine rootstocks may thus have survived in a potentially important number of plots which were abandoned early on and which subsequently underwent a transition to forest (Camerano & Terzuolo, 2015). As previously described by Jeger et al. (2016) in general and by Lessio et al. (2007) and Ripamonti et al. (2020) for Italy, gone-wild grapevines (GWGVs) which originated from abandoned vineyards or spontaneously grew from seeds dispersed by animals may constitute an undetected refuge for *S. titanus* and an uncontrolled FDp reservoir.

In this article, we continue this line of investigation by evaluating the possible role of vineyards abandoned early on which experienced a forest transition and might still host GWGVs which serve as a habitat for FDp vectors, as shown in Northwestern Italy by Rossi et al. (2019).

The aims of the study were to verify (i) the presence in Southern Switzerland of FDp-infected GWGVs in forest compartments that were previously vineyards as well as of FDp-infected GWGVs of spontaneous origin in forests located in the proximity of currently cultivated vineyards and (ii) the ability of the main (i.e., *S. titanus*) and the alternative (i.e., *O. ishidae*) FDp vectors to colonize and reproduce on GWGVs, forming a vector population that escapes the mandatory insecticide applications.

2 | MATERIALS AND METHODS

2.1 | Study area and experimental design

The study area consists of former (maintained until at least 1934) vineyard plots in Southern Switzerland (i.e., Canton Ticino and the Valle Mesolcina in Grisons, Figure 1a). This area is principally divided into two main regions, called Sopraceneri and Sottoceneri and differing in terrain geomorphology, population density (Cotti et al., 1990;

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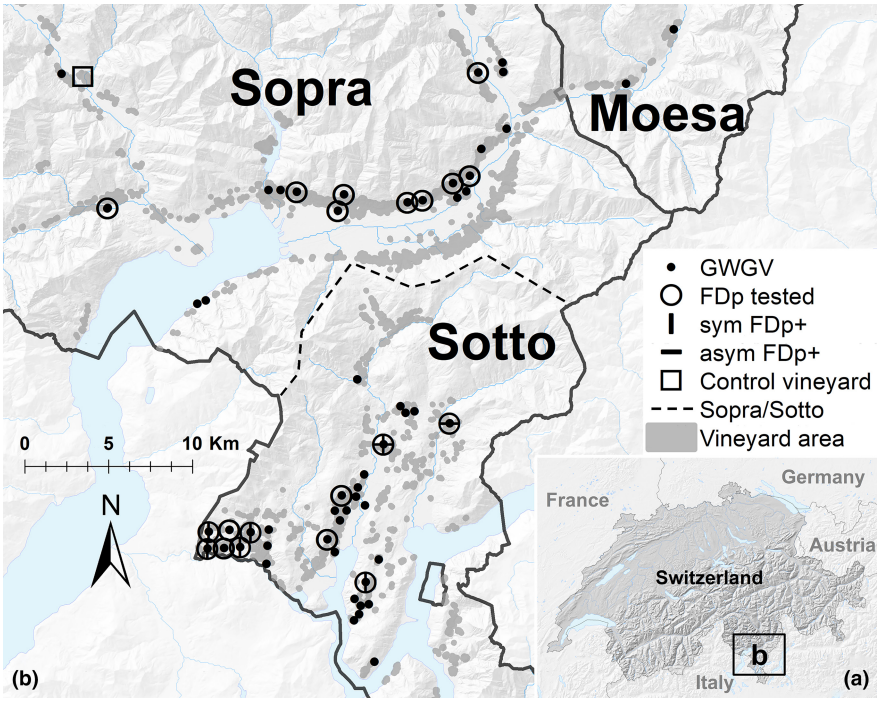

FIGURE 1 Location of the study area within Switzerland (a) and location of the confirmed sites hosting gone-wild grapevines in forest (GWGV, dots) and plots that were subjected to molecular analysis for Flavescence dorée phytoplasma detection (FDp, open circles; b)). The control vineyard is a cultivated vineyard free of Grapevine Yellows-related symptoms but with a sizeable population of *Scaphoideus titanus* (open square). Sym FDp+ = symptomatic and FDp-infected sample (vertical bar); asym FDp+ = asymptomatic and FDp-infected sample (horizontal bar). Sopra, Sopraceneri (Ticino); Sotto, Sottoceneri (Ticino); Moesa, Valle Mesolcina (Grisons).

Torricelli et al., 1997) and timing of the arrival and establishment of both *S. titanus* and FDp, which first colonized the Sottoceneri and only later the Sopraceneri region (Jermini et al., 2014). More precisely, *S. titanus* was first identified in Sottoceneri in 1967 by Baggiolini et al. (1968) and only 30 years later in Sopraceneri (Linder & Jermini, 2007), while FD *in planta* was again detected first in Sottoceneri and later in Sopraceneri, in 2004 and 2006, respectively (Jermini et al., 2014; Schaerer et al., 2007).

In 1934, the winegrowing area extended over 5′435 ha and progressively shrank to the current 1′143 ha (Krebs & Bertogliati, 2017; Wyler et al., 2022). The process of abandonment has mainly involved traditional marginal vineyards located on slopes and in close contact with the forest area. As a result, most of the formerly cultivated vineyards experienced a transition to forest (Ceschi, 2014).

In order to identify and locate potential long-abandoned vineyard sites, the vineyard signature drawn on the Swiss national maps of 1934, 1969, 1989 and 2007 was first digitized (as needed) and then intersected with the forest area signature of 2020 according to the large-scale topographic landscape model of Switzerland *swissTLM3D* (Swisstopo, 2021). The resulting intersections potentially represent former winegrowing areas which experienced a transition to the forest in the last decades but may still host GWGVs. In August 2021, a representative selection of the resulting spots was visited and searched for the presence of GWGVs, which was confirmed in 55 cases (Figure 1b). Among these, two additional plots were discovered in forest stands surroundings of the target spots during the field survey.

All the detected GWGV sites were described in terms of covered area, mean slope, distance from the nearest cultivated vineyard, forest type (including the presence of spontaneous plant species, such as *A. glutinosa*, *C. avellana*, etc., which may host FDp inoculum and/or alternative FDp vectors, such as *O. ishidae* [Mehle et al., 2019; Rizzoli et al., 2021]), last time of registration as vineyard on official topographic maps (hereinafter referred to as 'Last_T'), and presence of GWGVs showing symptoms linked to Grapevine Yellows (GYs) (EPPO/CABI, 1997). For the detected GWGVs which were not indicated on the map intersections, aerial images were checked to confirm the presence of forest since at least 1969 (Swisstopo, 2023), as well as to assess the possible origin of the grapevines.

Finally, 20 sites, 11 in the Sottoceneri and 9 in the Sopraceneri, were selected based on representativeness for the territory, accessibility (e.g., wild vegetation, slope), presence or absence of GY-symptomatic GWGVs (Figure 1b and Table 1), and presence of vineyards in the surroundings with known FDp-infected grapevines according to the routine surveys of the local Plant Protection Service in the last 5 years (data not shown). As a control for comparing the forest and the cultivated compartments for FDp vector abundance, the cultivated vineyard in the municipality of Maggia (Sopraceneri, Figure 1b; Table 1) was additionally selected since this area entered the perimeter of the mandatory insecticide applications only in 2021 (Fedlex, 2021). As a result, the control vineyard supported a sizeable population of *S. titanus* as confirmed by the ongoing surveillance run in collaboration with the Cantonal Plant Protection Service, which monitors the vector population from June to October by using yellow sticky traps (Rebell Giallo, Andermatt Biocontrol AG; YSTs) mounted on the highest wire of the training system (Table 1).

2.2 | Sample collection and preprocessing

Each of the 20 selected plots was revisited in August 2021 and a minimum of 12 randomly selected asymptomatic GWGV leaves were collected per plot, since rootstocks may be FDp-infected even when not showing any external symptoms (Caudwell et al., 1994; Eveillard et al., 2016).

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TABLE 1 Plot characteristics in terms of type of experiment (M = molecular analysis; T = traps for adult insects; H = hatching experiment), coordinates, region, mean slope, distance from nearest vineyard (DV), last year in which plot was registered as vineyard (Last_T); number of samples showing Grapevine Yellows symptoms over total number of collected samples (GY); number of positive samples resulted from molecular analysis for FDp detection on gone-wild grapevines over total number of analysed samples (positive samples: Cq < 35); number (Nr.) of yellow sticky traps (if applicable) and *Scaphoideus titanus* and *Orientus ishidae* captures per single trap; absolute (*N*) and specific (*N*/kg wood) hatching events of *S. titanus* and *O. ishidae* per plot.

									Yellow stick traps (YST)			Hatchings		
Plot ID	Type	Coordinates WGS 84	Region	Mean slope [degrees]	DV [m]	Last_T [y]	GY	FDp	Nr.	*S. titanus*/ YST	*O. Ishidae*/ YST	Leafhopper	*N*	*N*/kg wood
Gerra Piano_1	M, H	46°10′34.3″ N 8°54′10.9″ E	Sopraceneri	37.7	3	1969	0/1	0/1	-	-	-	*S. titanus*	2	0.51
												O. ishidae	0	0.00
Gordola_2	M, H	46°11′03.2″ N 8°52′09.9″ E	Sopraceneri	30.3	24	2007	0/1	0/1	-	-	-	*S. titanus*	4	2.40
												O. ishidae	1	0.60
Lamone_1	M, T, H	46°02′54.3"N 8°55′59.2″ E	Sottoceneri	29.7	5	1969	2/5	3/5	3	0.00	0.00	*S. titanus*	0	0.00
												O. ishidae	0	0.00
Losone_1	M, T, H	46°10′39.1" N 8°43′22.5″ E	Sopraceneri	29.2	270	2007	0/2	0/2	3	3.00	0.00	*S. titanus*	2	0.38
												O. ishidae	0	0.00
Monte Carasso_1	M, H	46°11′28.0" N 9°00′12.7″ E	Sopraceneri	36.9	3	2007	0/1	0/1	-	-	-	*S. titanus*	0	0.00
												O. ishidae	0	0.00
Medoscio_1	M, T, H	46°10′52.7" N 8°54′13.2″ E	Sopraceneri	30.2	26	2007	0/1	0/1	1	7.00	3.00	*S. titanus*	19	3.84
												O. ishidae	1	0.20
Monteggio_3	M, T, H	45°59′51.9" N 8°47′31.8″ E	Sottoceneri	26.0	63	1934	2/3	1/3	2	0.00	3.00	*S. titanus*	0	0.00
												O. ishidae	1	0.09
Monteggio_4	M, T, H	45°59′36.3" N 8°48′16.0″ E	Sottoceneri	25.4	9	1934	3/3	3/3	1	0.00	0.00	*S. titanus*	1	0.25
												O. ishidae	1	0.25
Monteggio_5	M, T, H	45°59′42.2" N 8°48′06.5″ E	Sottoceneri	20.3	21	1969	0/1	0/1	1	1.00	0.00	*S. titanus*	0	0.00
												O. ishidae	0	0.00
Sessa_1	M, T, H	46°00′00.9" N 8°49′18.2″ E	Sottoceneri	28.7	8	1969	1/1	1/1	1	0.00	0.00	*S. titanus*	2	0.25
												O. ishidae	3	0.37
Sonvico_1	M, T, H	46°03′30.6" N 8°59′03.7″ E	Sottoceneri	23.7	6	1934	0/1	1/1	3	0.66[a]	1.00	*S. titanus*	1	0.07
												O. ishidae	0	0.00
Cademario_1	M, T	46°01′15.9" N 8°53′59.1″ E	Sottoceneri	19.6	10	1989	0/1	0/1	1	0.00	0.00	-	-	-
Collina D'Oro_1	M	45°58′27.7" N 8°54′59.5″ E	Sottoceneri	16.3	140	1934	1/1	1/1	-	-	-	-	-	-
Gnosca_1	M, T	46°14′46.6" N 9°00′47.0″ E	Sopraceneri	4.5	300	Forest[b]	0/1	0/1	1	0.00	0.00	-	-	-
Gudo_1	M, T	46°10′39.7" N 8°57′17.8″ E	Sopraceneri	24.2	8	1969	0/1	0/1	1	1.00	1.00	-	-	-
Monte Carasso_2	M	46°11′22.0" N 8°59′38.7″ E	Sopraceneri	44.8	6	1934	0/1	0/1	-	-	-	-	-	-
Monteggio_1	M, T	45°59′45.3" N 8°48′04.3″ E	Sottoceneri	17.5	12	1969	0/1	0/1	1	0.00	0.00	-	-	-

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TABLE 1 (Continued)

Plot ID	Type	Coordinates WGS 84	Region	Mean slope [degrees]	DV [m]	Last_T [y]	GY	FDp	Yellow stick traps (YST)			Hatchings		
									Nr.	*S. titanus*/ YST	*O. ishidae*/ YST	Leafhopper	N	N/kg wood
Monteggio_2	M, T	45°59′47.2″N 8°47′50.0″E	Sottoceneri	23.7	25	1934	1/2	1/2	1	0.00	0.00	-	-	-
Sementina_1	M, T	46°10′42.9″N 8°58′00.1″E	Sopraceneri	27.0	50	Forest[b]	0/1	0/1	1	1.00	1.00[c]	-	-	-
Vernate_1	M, T	45°59′51.5″N 8°53′17.1″E	Sottoceneri	15.5	270	1934	0/1	0/1	1	0.00	1.00	-	-	-
Maggia (control)	M, T, H	46°14′54.2″N 8°42′20.4″E	Sopraceneri	5.3	0	2023	0[d]	NA[d]	6	85.00[e]	7.17[e]	*S. titanus*	199	51.00
												O. ishidae	2	0.26

[a]One single FDp-infected *S. titanus* specimen.
[b]Never mapped as vineyard and visible as forest since 1969 on aerial images.
[c]One single FDp-infected *O. ishidae* specimen.
[d]No symptomatic samples over around 1000 grapevines. No molecular analysis performed.
[e]Referred to captures from June to October 2021.

Where present (i.e., six plots), all visible GY-symptomatic GWGV leaves were additionally or exclusively collected (see plots indicated with type 'M' in Table 1). The collected leaf material was immediately transported to the research facilities, where the petioles and the midribs were excised and frozen at −20°C.

During the same period, one to three YSTs were mounted in a subset of 15 plots (type 'T' in Table 1; Figure 2a) with the aim of assessing the presence and FDp infection status of *S. titanus* and *O. ishidae*. YSTs were mounted on a pole at 1.20–1.50 m above ground and were exposed from August until the end of October and changed at an interval of 2 weeks. Target insect species were then determined in the lab using a stereo microscope (Olympus SZX16 with SDF PLAPO 1XPF objective lenses) and by consulting the determination keys provided by Della Giustina et al. (1992) and Günthart and Mühlethaler (2002). Identified specimens were then detached from the YSTs using Glurex forte (50%–100% D-Limonene [v/v], Andermatt Biocontrol AG), washed in 70% Ethanol (v/v), transferred into tubes containing 99% Ethanol (v/v) and frozen at −20°C.

For the hatching experiment, 1.7–14.3 kg of woody vines with an age of at least 2 years were collected in November 2021 at heights ranging from 0 to 10 m from a subset of 11 plots (type 'H' in Table 1; Figure 2b). Vine samples were then reduced to 30 cm in length, grouped into bundles and stored in a cold chamber (T = 5°C, relative humidity = 55%). Pruned canes from the cultivated vineyard of Maggia were sampled as a positive control.

2.3 | Nucleic acid extraction and molecular analysis

2.3.1 | Leaf samples

Petioles and midribs from 3 to 4 different leaves per specimen, equivalent to 0.5 to 1 g of plant material, were ground in 6 mL of extraction buffer (3% Cetyltrimethylammonium bromide CTAB, 1.4 M NaCl, 25 mM EDTA, 100 mM Tris, pH 8.0) using a Homex grinder (Bioreba). Subsequently, 2 mL of this homogenate was centrifuged for 10 min at 1′000× g. 900 µL of the supernatant were mixed with 2 µL of β-Mercaptoethanol and shaken for 30 min at 600 rpm and at 65°C. Chloroform/Isoamylalcohol (900 µL) was added, homogenized by vortexing for 5 s and centrifuged for 5 min at 3′000× g. The aqueous layer was carefully transferred to a new tube, mixed with an equal volume of cold isopropanol and incubated for 30 min at −20°C for DNA precipitation. Precipitated material was recovered by 2 min of centrifugation at 10′,000× g and washed with 1 mL of 70% Ethanol. The DNA pellets were dried overnight at room temperature and resuspended into 100 µL of PCR-grade water.

2.3.2 | Insect samples

Insects were homogenized in 900 µL of extraction buffer (3% Cetyltrimethylammonium bromide CTAB, 1.4 M NaCl, 25 mM EDTA,

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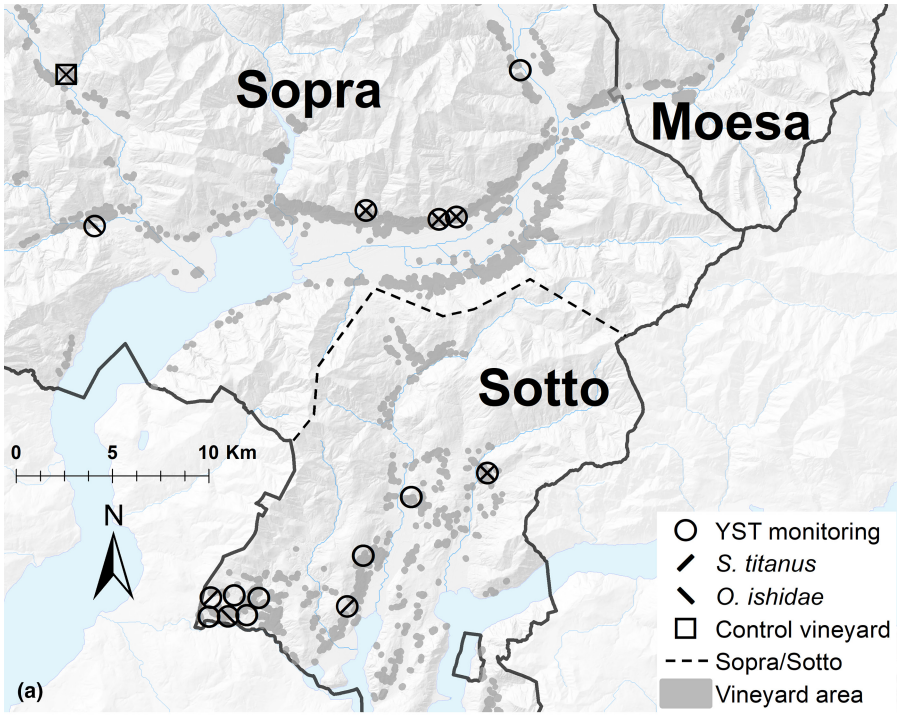

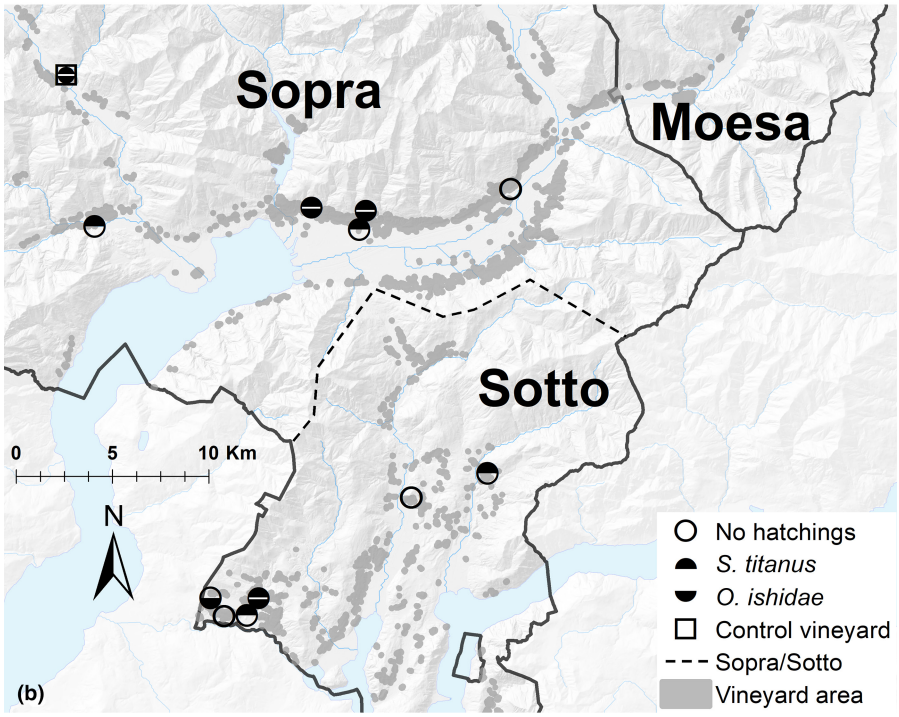

FIGURE 2 Distribution and qualitative results for the insect vectors. Presence or absence of *Scaphoideus titanus* (diagonal line bottom left to top right) and/or *Orientus ishidae* (diagonal line top left to bottom right) for the 15 gone-wild grapevines plots monitored with yellow sticky traps (YSTs; open circles, a)) Presence and absence of *S. titanus* (top half-moon) and/or *O. ishidae* (bottom half-moon) for the 11 plots subjected to the hatching experiment (open circles; b)). The control vineyard is a cultivated vineyard free of Grapevine Yellows-related symptoms but with a sizeable population of *S. titanus* (open square). Sopra, Sopraceneri (Ticino); Sotto, Sottoceneri (Ticino); Moesa, Valle Mesolcina (Grisons).

100 mM Tris–HCl, 2% β-Mercaptoethanol, pH 8.0) and shaken for 30 min at 600 rpm and 65°C. 900 µL of Chloroform/Isoamylalcohol were added, homogenized by vortexing for 5 s and centrifuged for 5 min at 3′000× g. The aqueous layer was carefully transferred to a new tube, mixed with an equal volume of cold Isopropanol, and incubated for 60 min at −20°C for DNA precipitation. Precipitated material was recovered by 2 min of centrifugation at 10′,000× g and washed with 1 mL of 70% Ethanol. The DNA pellet was dried overnight at room temperature and resuspended into 100 µL of PCR-grade water.

2.3.3 | FDp detection

For FDp detection in the leaf samples, a triplex qPCR method according to Pelletier et al. (2009) was applied with a final volume of 15 µL using a GoTaq Probe qPCR kit (Promega) and CFX96 thermocycler (Bio-Rad). The thermal cycle involved a denaturation phase of 5 min at 95°C for Hot Start Taq DNA polymerase activation, followed by 40 cycles of 15 s at 94°C and 30 s at 60°C. All leaf samples were also analysed for the presence of '*Candidatus* Phytoplasma solani', the pathogenic agent associated with 'Bois noir'.

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To assess the presence of FDp in insects, a duplex qPCR method with the simultaneous detection of FDp (as in Pelletier et al., 2009) and the Cytochrome Oxydase I (COI) of the insects (Appendix S1) was applied with a final volume of 15 µL using a GoTaq Probe qPCR kit (Promega) and CFX96 thermocycler (Bio-Rad) and the same cycling conditions as above.

All the FDp-infected GWGV and insect samples had a Cq value lower than 35.

2.4 | Hatching experiment

Since the direct counting of eggs inserted under the bark usually leads to egg destruction making it difficult to ensure species determination, the indirect method of considering freshly hatched nymphs was chosen (Chuche & Thiéry, 2009).

To this purpose, in April 2022, each collected wood bundle corresponding to a sampling site (i.e., 11 GWGV sites and one vineyard control site; see type 'H' in Table 1) was removed from the cold chamber and placed in a rearing cage (160 µm nylon mesh, 120 × 50 × 50 cm, BugDorm, MegaView Science Co. Ltd.). Two potted young grapevine plants (directly grown from locally obtained seeds of 'Merlot' cv.) were then added to each cage as nourishment sources for the nymphs which were expected to hatch from the deposited eggs. To avoid egg desiccation, the wood was moistened every other day, and potted grapevines were watered once a week. The air temperature of the experimental room was recorded every 2 h by means of a data logger (HOBO Pro v2 U23-001, Onset Computer Corporation).

Cages were inspected once every 3 days from the start of the experiment up to the observation of the first hatched nymph. Subsequently, the inspection took place daily from Monday to Friday until the end of June and 3 days a week in July (Monday, Wednesday and Friday). After the last hatching occurred, the cages were inspected for an additional week to confirm the end of the hatching phase.

Potential newly hatched *S. titanus* and *O. ishidae* nymphs were sampled with an electric aspirator (InsectaVac Aspirator, BioQuip Products Inc.), classified in the same way as was previously described for the specimens caught on YST and eventually transferred into two additional rearing cages (one for each species) provided with two potted grapevines for checking the potential of further development of the insects up to the adult stage.

2.5 | Statistical analysis

The embryonic development was calculated as the time needed for egg hatching based on degree-days (DDs) and expressed as days for convenience. DDs were calculated according to the formula:

$$DD = \sum_{i=1}^{n} \frac{\max[0, (T_i - T_b)]}{12},$$

where n is the number of considered hours since 1 January, T_i is the temperature measured by the datalogger at hour i (12 readings per day; for the time before the start of the experiment, T_i is 5°C) and T_b is the base developmental threshold temperature for *S. titanus*, which was set to 5°C as proposed by Chuche and Thiéry (2009).

Hatching dynamics of *S. titanus* were evaluated by means of an inverse transformation of the Kaplan–Meyer survival curve and compared with the log-rank and Gehan–Wilcoxon (with Peto modification) tests (Chuche & Thiéry, 2009; Pyke & Thompson, 1986). Dynamics of the single GWGV plots were compared individually with the control and between each other. Daily hatching variations over time were compared with a Spearman correlation test. The correlation between Last_T and FDp-infection status was calculated with a bias-corrected CramerV test (Cramer, 1946).

All the statistical analyses were performed using the software R (version 4.2.2; R Core Team, 2022). ArcGIS (release 10.6.1; ESRI, 2011) was used for spatial analysis and mapping renditions.

3 | RESULTS

3.1 | Distribution of symptomatic and FDp-infected GWGVs

In the Sottoceneri region, 7 of the 11 surveyed plots hosted FDp-infected GWGVs (Cq < 35). More precisely, six plots hosted symptomatic FDp-infected GWGVs, one plot hosted both symptomatic and asymptomatic FDp-infected GWGVs (Lamone_1), and one plot hosted asymptomatic leaf material which was nevertheless FDp-infected (Sonvico_1; Figure 1b; see plots with type 'M' in Table 1). On the other hand, none of the nine considered plots in the Sopraceneri region hosted either symptomatic or FDp-infected GWGVs. Last_T and FDp-infection status of the GWGVs originating from abandoned vineyards were not significantly correlated ($v = 0.6$, $\chi^2 = 6.4$, $p > 0.05$, $n = 18$).

3.2 | Captures on YSTs and egg-hatching counts

S. titanus individuals were captured in 6 of the 15 GWGV plots monitored with YSTs in 2021. *O. ishidae* could also be detected in six plots, whereby the plots hosting *O. ishidae* were however not entirely identical to the plots hosting *S. titanus* (see plots with type 'T' in Table 1; Figure 2a). Overall, a total of 21 *S. titanus* and 15 *O. ishidae* specimens were captured, of which one *S. titanus* individual at the site Sonvico_1 and one *O. ishidae* individual at the site Sementina_1 resulted infected by FDp (Table 1).

A total of 31 *S. titanus* nymphs were collected from 7 of the 11 GWGV plots (64%) considered for the hatching experiment, corresponding to a newly hatched nymph density ranging from 0.07 to 3.84 per kg of fresh wood (see plots with type 'H' in Table 1; Figure 2b). Five plots (46%) also hosted *O. ishidae* (7 nymphs overall). In three plots, no nymphs of either FDp vectors were observed.

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Finally, 199 *S. titanus* and two *O. ishidae* nymphs were collected from the cage hosting the pruned canes of the control vineyard.

From a spatial point of view, there is no significant correlation between the distance of a GWGV to the nearest cultivated vineyard (which may be up to 270 m as is the case for Losone_1) and the presence of newly hatched *S. titanus* nymphs ($n = 11$, $r_S = 0.30$, $p > 0.05$; Table 1). However, there is a general correspondence between the plots in which adult insects were captured in 2021 by means of YSTs and the plots which yielded egg hatchings in 2022, except for Monteggio_5, where only one adult individual of *S. titanus* was caught in 2021 and no nymphs of either *S. titanus* or *O. ishidae* were later observed during the hatching experiment in 2022 (Figure 2a,b). Hatching events of both insect species are independent of the presence of GY-symptomatic and/or FDp-infected GWGVs observed in the previous year (Table 1).

In total, 50 of the 211 nymphs which were collected and determined to be *S. titanus* individuals (i.e., 23.7%; 19 nymphs did not survive sampling and transfer to the final rearing cage) were able to develop to the adult stage. Males were 1.4 times more abundant than female specimens (i.e., 58% male, 42% female). On the other hand, none of the nine *O. ishidae* nymphs (two nymphs died at sampling) reached the adult stage.

3.3 | Egg-hatching dynamics

Due to the low number of hatched nymphs in most of the GWGV plots (see plots with type 'H' in Table 1), *S. titanus* hatching observations from the single GWGV plots were grouped for the comparison of the hatching dynamics with the control vineyard.

Hatching started 42 days after removing the wood samples from the cold room for the control plot and 5 days later for the GWGV plots. The accumulated DDs at the first hatching event were 785.06 and 875.66 for the control and the GWGV plots, respectively. The overall egg-hatching period was 21 days (512 DD) longer for the control. However, the final part of the hatching period, which constitutes this additional time period, contributed to only 2% (four nymphs) of the total hatchings (Table 2). The mean embryonic development time and the mean accumulated DD at hatching were comparable between the two groups (i.e., $p > 0.05$).

Similarly, the hatching dynamics based on the inverted Kaplan–Meyer survival curve for the two categories were not significantly different (log-rank: $\chi^2 = 0.7$, Gehan–Wilcoxon: $\chi^2 = 0.7$, $p > 0.05$, Figure 3). Moreover, the proportions of 25% and 50% of the total hatchings were reached after having accumulated ca. 940 DD (8 days after the first hatching) and ca. 1075 DD (14 days after the

TABLE 2 Embryonic development time of *Scaphoideus titanus* expressed as degree-days (DDs) and days for gone-wild grapevines (GWGV) and the control.

		Degree-days				Days			
Category	*N*	ED_{dd_min}	ED_{dd_max}	$ED_{dd\_mean} \pm SE$	DDA	ED_{t_min}	ED_{t_max}	$ED_{t\_mean} \pm SE$	HD
GWGV	31	875.66	1724.96	1189.57 ± 45.86	849.29	47	84	60.90 ± 2.01	37
Control	199	785.06	2146.23	1135.34 ± 16.83	1361.17	42	100	57.89 ± 0.73	58

Abbreviations: DDA, DDs accumulated between first and last hatching; ED_{dd_mean}, mean accumulated DDs; ED_{dd_min} and ED_{dd_max}, DDs accumulated at the first and last hatching, respectively; ED_{t_mean}, mean time (days) of embryonic development; ED_{t_min} and ED_{t_max}, minimal and maximal time (days) of embryonic development, respectively; HD, hatching period duration in days; *N*, number of hatched nymphs; SE, standard error.

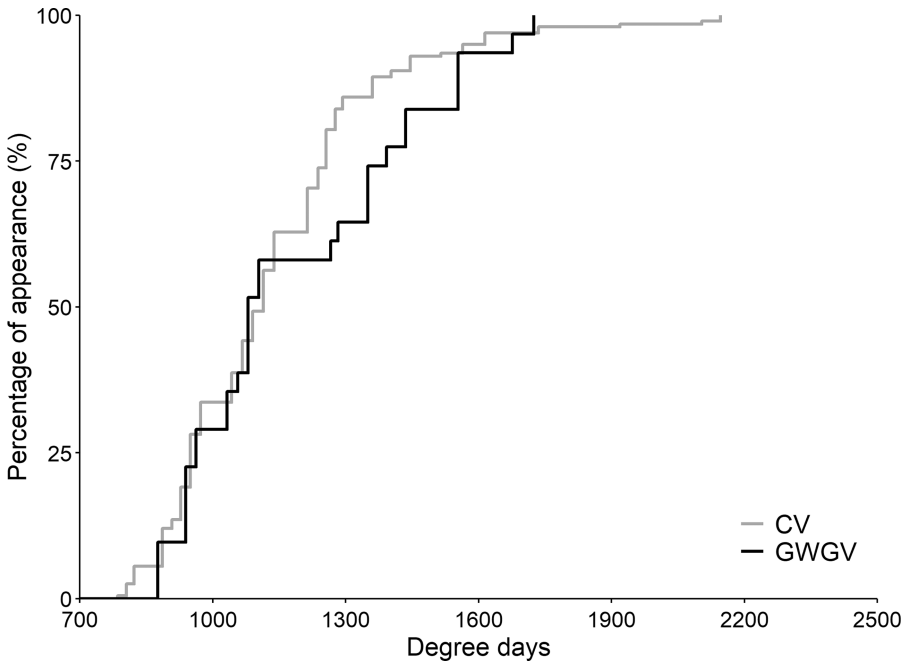

FIGURE 3 Cumulative percentage of *Scaphoideus titanus* hatchings by degree-day (inverted Kaplan–Meyer) for the pooled gone-wild grapevines plots (GWGV, black line) and the cultivated vineyard (CV, control, grey line).

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first hatching for both the control and the GWGV plots). After ca. 60% of the total hatchings was reached, the relative GWGV hatching rate decreased such that 75% was attained ca. 100 DD later than the control (i.e., at 1240 DD or day 20 for the control, compared with 1350 DD or day 26 for the GWGVs). Finally, the hatching period in the GWGV plots ended 16 days earlier compared with the control.

The daily variations in hatching rate over time were significantly correlated ($n=35$, $r_S=0.45$, $p<0.01$, Figure 4), and the peak was reached 8 days after the first hatching for both the control and the GWGV plots.

4 | DISCUSSION

4.1 | GWGVs as habitat for FDp vectors

The results confirm the potential role of GWGVs growing in forests as a suitable habitat for the main and alternative FDp vectors *S. titanus* and *O. ishidae* (Chireceanu, 2014; Lessio et al., 2007, 2016; Pavan et al., 2012; Ripamonti et al., 2020).

Although showing differences in frequencies and abundances, both vectors were observed as adults on-site and as nymphs in the hatching experiment. In the specific cases where no adult *S. titanus* specimens were captured using YSTs (e.g., Lamone_1, Table 1), it may be assumed that the sampling effort was insufficient in terms of number and location of the YSTs, as well as the time of placement (after the population's peak). As for the hatchings, especially for GWGVs, the wood used for the experiment was sampled in a heterogeneous context where it is not possible to target specific parts of the grapevine as is the case in cultivated vineyards (pruned canes). Nevertheless, considering that even in plots with no adult captures and/or hatchings (i.e., Lamone_1, Monteggio_3, Sessa_1, Table 1) FDp-infected GWGVs were detected, we assume that *S. titanus* may even be present in these cases.

As for the hatching experiment and in particular for *S. titanus*, considering that hatching event counting is an indirect method that lacks the indication of egg mortality (including desiccation), we can even assume that the actual oviposition rate was most likely underestimated (Bagnoli & Gargani, 2011). Nevertheless, newly hatched nymphs were observed for 64% of the investigated plots, confirming the widespread colonization of the wild compartment by *S. titanus*. The colonization of such compartments (up to 63 m and 270 m from the nearest cultivated vineyard in Sottoceneri and Sopraceneri, respectively) may be due to multiple, non-exclusive factors: *S. titanus* specimens found on GWGVs which originated from vineyards which were abandoned relatively recently may be the result of leafhopper populations which survived the transition from cultivated vineyard to forest on non-eradicated vines which later became wild. Considering that the report of the presence of *S. titanus* in Canton Ticino dates back to 1967 and 1998 for the Sottoceneri and Sopraceneri regions, respectively (Baggiolini et al., 1968; Linder & Jermini, 2007), it may be assumed that residual *S. titanus* populations established in GWGV plots in Sottoceneri may date back several decades. The presence of *S. titanus* on GWGVs may however also be the result of the active migration from the nearest cultivated vineyard as is known to occur in its native range in North America (Maixner et al., 1993) and has already been shown by Lessio et al. (2007) and Chireceanu (2014) to occur in Italy and Romania, respectively. In this regard, the frequent proximity of the vineyards to the forest edge, as is for instance the case in the study area (Wyler et al., 2021), may additionally facilitate the migration from one compartment to the other, as has already been shown in general for abandoned vineyards in the bordering Piedmont in Italy (Lessio et al., 2007).

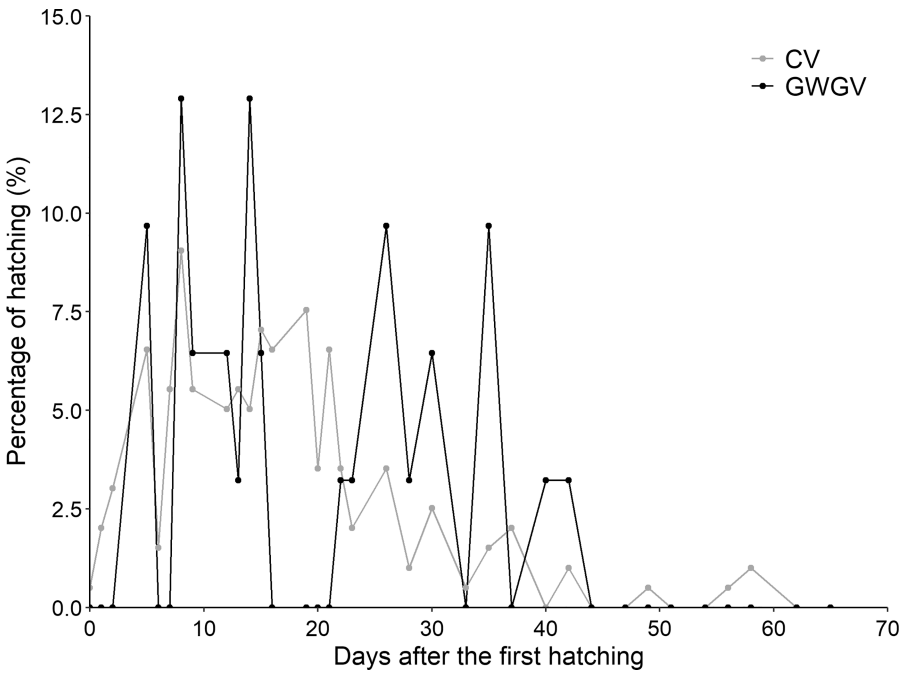

FIGURE 4 Daily egg-hatching dynamics of *Scaphoideus titanus* for the pooled gone-wild grapevines plots (GWGV, black line) and the cultivated vineyard (CV, control, grey line).

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4.2 | Potential epidemiological role of GWGVs

The results demonstrate that the FDp inoculum and populations of both the main and the alternative FDp vectors may well be maintained in the entire wild compartment, including the forest. These vector populations are usually not actively monitored and thus remain undetected. Moreover, at least in the specific case of Switzerland, since insecticide applications are strictly prohibited in forests (Fedlex, 1991), such populations (especially those of *S. titanus*) will also remain uncontrolled in the future. Consequently, they may well have an epidemiological impact, considering the possible migration to the cultivated compartment (Lessio et al., 2014). The concomitant presence of *O. ishidae* may reinforce this role in the maintenance or triggering of the FD epidemics, especially in combination with alternative FDp host plant species such as *Alnus glutinosa*, on which infection rates up to 85% were found for *O. ishidae* (Rizzoli et al., 2021). The co-habitation of GWGVs and other plant species known to be potentially infected with FDp genotypes compatible with *S. titanus* and *O. ishidae* represents an additional risk factor for the FD epidemics (Malembic-Maher et al., 2020; Rizzoli et al., 2021). The same may be true for other alternative FDp epidemiological cycles, such as the association of *Dictyophara europaea* (Linnaeus, 1767) with *Clematis vitalba* (Filippin et al., 2009), although, contrarily to *O. ishidae*, *D. europaea* has never been found to be able to lay eggs on grapevine (Krstić et al., 2016).

The potential epidemiological role of GWGVs in the forest is further stressed by the fitness of the hatching nymphs of *S. titanus*, which apparently show the same capability of development as the ones originating from cultivated vineyards. This is confirmed by the correlation between the daily hatching dynamics, including the peak of hatching occurring on the same day for both the GWGV plots and the control. Moreover, the high-mean temperature recorded in the nursery during the hatching period between 1 June and 29 July 2022 (28.4 ± 0.2°C; *mean ± standard error*) probably partially hindered the embryonic development, as demonstrated by Falzoi et al. (2014), who reported a mortality rate of 99.60% and 97.56% at the constant temperatures of 28°C and 29°C, respectively.

The different contexts of oviposition (forest vs. cultivated vineyard) do not seem to be a key factor which influences the hatching dynamics (Chuche & Thiéry, 2009), suggesting full synchrony between the vector populations on GWGVs and grapevine phenology, as was already shown in cultivated vineyards, regardless of the conditions during diapause and grapevine cultivar (Chuche et al., 2015). This eventually increases the probability of survival of *S. titanus* in case of migration from GWGVs in forests and from the wild compartment in general to a cultivated vineyard (Lessio et al., 2014).

In addition, the ability of some *S. titanus* nymphs collected during the experiment to complete the development to the adult stage under the prohibitive average temperatures experienced during the hatching period indicates the potential for this leafhopper to adapt to rising temperatures (Falzoi et al., 2014). In light of the ongoing climate change, this may imply a risk of colonization of new regions hitherto untouched by the FD epidemics (Jeger et al., 2016; Quiroga et al., 2017; Sneiders et al., 2019).

The fact that FDp-infected GWGVs were detected exclusively in the Sottoceneri region may be related to the time since the full establishment of the three main components of the FD epidemiological cycle (i.e., FDp, *S. titanus*, and grapevines, including GWGVs as host plant), as well as the time needed for the transition of abandoned vineyards to forest, which generally occurred earlier in Sottoceneri according to the map intersections, despite the lack of significance reported by the Cramer correlation. Nevertheless, it may be assumed that FDp-infected GWGVs may also be present in the Sopraceneri region, considering the widespread presence of FDp-infected vineyards and residual pockets of *S. titanus* populations that survive the application of insecticides (Canton of Ticino, 2020; Jermini et al., 2014). Moreover, secondary FDp epidemiological cycles including alternative vectors and host plant species, such as *O. ishidae* and *A. glutinosa*, may also trigger an infection in GWGVs directly from the surrounding forest (Malembic-Maher et al., 2020; Rizzoli et al., 2021).

These considerations show that GWGVs and vector populations in the wild should play a pivotal role in future FDp epidemiology and risk assessment.

5 | CONCLUSION

In forests, gone-wild or spontaneously grown (i.e., by reproductive propagation of seeds dispersed by animals) grapevines may act as a habitat for *S. titanus* and *O. ishidae*, the main and alternative vectors of phytoplasmas associated with the FD grapevine disease. Such grapevines represent a potential source of FDp inoculum which may be transferred to and from cultivated vineyards when not properly controlled, that is to say, rogued (Ripamonti et al., 2020). In this regard, the lack of control regarding the rogueing of GWGVs in general, and of FDp-infected GWGVs in particular, represents a clear flaw in the control strategy of the FD epidemics (Jeger et al., 2016; Ripamonti et al., 2020).

Further research is needed to assess the abundance and the infection rate of FDp vectors and GWGVs in the forest compartment, as well as to identify the involved FDp genotypes (Malembic-Maher et al., 2020). In general, abandoned vineyards and GWGVs should be rapidly identified and rogued to lower the presence of FDp inoculum and remove alternative habitats for the FDp vectors (*S. titanus* in particular) for both phytosanitary and economic reasons (Jeger et al., 2016; Lessio et al., 2007; Ripamonti et al., 2020). Eventually, such measures may minimize the possible flow of FDp between the wild and the cultivated compartments.

AUTHOR CONTRIBUTIONS

Alan Oggier: Conceptualization; methodology; data curation; investigation; formal analysis; visualization; writing – original draft. **Marco Conedera:** Writing – review and editing; funding acquisition; project administration; supervision. **Mauro Jermini:** Writing – review and

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editing. **Christophe Debonneville:** Investigation; validation; writing – review and editing. **Olivier Schumpp:** Writing – review and editing; funding acquisition. **Attilio Rizzoli:** Conceptualization; methodology; writing – review and editing; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data supporting the reported results can be found at https://www.doi.org/10.16904/envidat.385.

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