

A survey of potential vectors of the plant pathogenic bacterium *Xylella fastidiosa* in the Basilicata Region, Italy

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Abstract

The plain along the Ionian coast and the Matera hills are close to the border of the Apulia region, where, since 2013, the bacterium *Xylella fastidiosa* has been causing the death of olive trees. During 2016-2018 a study was conducted on the potential insect vectors of *X. fastidiosa* in eight olive groves, located in three areas of the province of Matera (Basilicata, Italy), characterized by different agricultural landscapes. We captured three spittlebug species, *Philaenus spumarius* (L.), *Neophilaenus campestris* (Fallen) and *Lepyronia coleoptrata* (L.), in addition to the cicadellid *Cicadella viridis* (L.). Among them, *P. spumarius* was by far the most prevalent species in 2016, while in 2017 and 2018 catch rates were modest for all the candidate vector species. The population trend of the four insect species in the June-October period was reported about the three study areas in the years considered.

Key words: olive, Auchenorrhyncha, spittlebugs, *Cicadella viridis*, vectors.

Introduction

Xylella fastidiosa subspecies *pauca* (CoDIRO strain) is the pathogen responsible for the ‘olive quick decline syndrome’ (OQDS) reported for the Apulia region, Italy, since 2013 (Saponari *et al.*, 2013). Several models, essentially based on climatic parameters, have shown that the potential distribution of this pathogen comprises Portugal, Spain, Italy, France, Albania, Montenegro, Greece and Turkey as well as all countries of northern Africa and the Middle East (Bosso *et al.*, 2016a; Schneider *et al.*, 2020). After the detection of *X. fastidiosa* in Italy, surveys in European countries revealed the presence of this bacterium species (including the subsp. *pauca*, *multiplex*, and *fastidiosa*) in France, Spain, and Portugal (Olmo *et al.*, 2021). However, the genotypes of *X. fastidiosa*, detected on olive trees, were different to that detected in the Apulia region, where all isolates harboured the subspecies *pauca*, sequence type ST53 (Loconsole *et al.*, 2016). Indeed, a new sequence type of the subspecies *pauca* (ST80) was related to the olive decline in Ibiza while the subspecies *multiplex* was associated with wild olive trees in Menorca (Olmo *et al.*, 2021).

Considering the risk that *X. fastidiosa* may cross the current boundaries, studies on potential vectors and olive tree monitoring were performed (Morente *et al.*, 2018; Cornara *et al.*, 2019; Kalaitzaki *et al.*, 2019; Antonatos *et al.*, 2020). The interest in these studies arises from the fact that the list of potential vector species and their population level can be widely affected by several ecological factors.

X. fastidiosa is a xylem-inhabiting bacterium, exclusively transmitted by xylem sap-feeding Auchenorrhyncha (Hill and Purcell, 1995; Redak *et al.*, 2004), all of which are considered as potential vectors (Purcell, 1989). In the Salento area (southern Apulia region), the first area where the OQDS was observed, four xylem feeding species were collected (Cornara *et al.*, 2017a): *Philaenus spumarius* (L.) (Hemiptera Aphrophoridae), *Neophilaenus campestris* (Fallen) (Hemiptera Aphrophoridae),

Cercopis sanguinolenta (Scopoli) (Hemiptera Cercopidae) and *Cicada orni* L. (Hemiptera Cicadidae). As shown by a recent study, *C. orni* may either have a minor role or no role in the natural spread of *X. fastidiosa* (Cornara *et al.*, 2020). *N. campestris*, present at very low densities in the Apulia region, equally played a marginal role as a vector (Dongiovanni *et al.*, 2019). Moreover, *N. campestris* adults are basically associated with conifers, which are preferred to olive tree (Bodino *et al.*, 2020). *P. spumarius* is the only abundant species showing high rates of *X. fastidiosa* infection (Cornara *et al.*, 2017a; 2017b) and it can transmit the bacterium to host plants from May to October. Cornara *et al.* (2018) provided a comprehensive state of the art analysis about this species. Another spittlebug species, *Philaenus italosignus* Drosopoulos et Remane, was recorded in central and northern Apulia region (Cavaliere *et al.*, 2019) and in other Italian regions (Panzavolta *et al.*, 2019). Transmission experiments showed that this spittlebug is a competent vector of *X. fastidiosa* (Cavaliere *et al.*, 2019), and it may play an important future role in the spread of this bacterium. Currently, final data on vector competence are available only for the CoDIRO strain of *X. fastidiosa* (EFSA, 2018).

In Italy, *X. fastidiosa* ST53 has spread northward from the first detection area, remaining apparently confined to the Apulia region until 2020 (Official Bulletin of Apulia region, 2020). However, in March 2018, the presence of infected olive trees was reported in the agricultural area of Taranto, on the border with the Basilicata Region (Official Bulletin of Apulia region, 2018).

According to the maximum entropy model proposed by Bosso *et al.* (2016b) for the potential geographical distribution of *X. fastidiosa* in Italy, in Basilicata about 30% of the regional surface area is potentially highly suitable for this bacterium. The plain of the Ionian coast and the Matera hills, close to the border of the Apulia region, fall within the area that, according to the model above, has very favourable conditions to the pathogen. The greatly

variable agricultural landscape in the Matera area might affect the number of potential insect vectors, their population dynamics and their tendency to host olive trees. Indeed, in different areas, the faunistic composition of spittlebugs, their phenology and seasonal abundance can be quite different (Bodino *et al.*, 2019). The knowledge of ecological factors affecting the insect vectors in areas other than the one currently affected by the disease may be important for the containment of *X. fastidiosa* spread. This three-year study aimed to provide a survey of the potential insect vectors of *X. fastidiosa* in the Matera area and their population dynamics in olive groves.

Materials and methods

Sampling sites

Samplings were carried out in eight olive groves in three different areas of the province of Matera, Basilicata Region, Italy: the hill of Ferrandina (3 olive groves; Ferr1, 40°29'14.1"N 16°27'52.2"E; Ferr2, 40°30'05.6"N 16°27'54.9"E; Ferr3, 40°29'06.7"N 16°28'57.8"E), at the bottom of the hill of Bernalda (2 olive groves; Bern1, 40°24'39.6"N 16°39'52.3"E; Bern2, 40°24'00.3"N 16°41'43.2"E) and the plain of Metaponto (3 olive groves; Met1, 40°24'46.7"N 16°48'11.2"E; Met2, 40°25'21.1"N 16°46'53.1"E; Met3, 40°25'12.2"N 16°49'12.3"E). The olive groves represent the variability of the conditions within the three areas of this study.

The area of Metaponto is the closest one to the border of the Apulia region. The terrain is flat. The olive groves are very variable in size and are surrounded by other orchards, vegetable farms or uncultivated land. The sampled olive groves reflected this area's typical variability in size: Met1 3.4 ha, Met2 1 ha and Met3 0.6 ha. The olive trees of these farms, cultivar Frantoio, were 10-15 years old, with a regular planting layout of 5 or 6 m. In addition to the young olive trees covering about 0.7 ha, Met2 had a strip of old trees with 12 m planting layout. Out of the three olive groves, Met3, located in a depressed area, was the wettest. Soil management in the Metaponto plain, as in the other two study areas, was useful to reduce fire risks. The soil was tilled on April-May or left covered with grass until the end of June, when the grass was cut. However, soil tillage left irregular patches of grass in various points of the olive grove, especially at the base of trees. In Met2 the young olive grove was abandoned and no cultivation techniques were applied, while in the strip with old trees that belonged to a different owner, the soil was tilled. Tillage was also applied in Met1. In Met 3 the soil was kept grassed.

In the area at the bottom of the hill of Bernalda, the soil tends to be more moist than in the Metaponto area and with a soil moisture gradient determined by the ground slope. The varying size olive groves are close to uncultivated areas that include extensive shrublands, quite rich in Mediterranean maquis. Bern1 is 4 ha in size. The olive grove borders on two sides a large Mediterranean scrub area, rich in shrubs, on the third side it borders with an herbaceous overgrown where the *Dittrichia viscosa* (L.) prevails, while it borders a road on the fourth side. The land has 5% ground slope. In the highest part, on an area

of 0.6 ha, there are centennial trees, cv Ogliarola del Bradano, with a highly irregular planting layout (6-16 m). In this part of the olive grove the soil is left with spontaneous grassing. The remaining surface consists of young trees (10-15 years old) of the cvs Frantoiana, Leccino and Nociara in the middle of which there are centennial trees of the cv Ogliarola del Bradano with fairly regular planting distances. The planting layout is 5 m. In this part of the olive grove, the soil is tilled. The second olive grove sampled in this area, Bern 2, has an area of 1.4 ha. The olive grove is flat, in a depressed position below the road it borders on one side, and the Bernalda hill, mostly uncultivated. The trees are centennials, of the cv Ogliarola del Bradano: they have an irregular planting layout (7-18 m). The ground is left with grass.

The hill of Ferrandina is olive monoculture. The entire hill is completely covered with olive groves and the Mediterranean shrub is mainly present in the access roads or along some strips that separate two areas with a big height difference. The olive trees are all centennials, almost exclusively cv Maiatica, with a large and irregular planting layout. Ferr1 has an area of 5 ha, with a slope of 5%. During this study the half olive grove was kept grassed and the soil was tilled, in the other half. Ferr2 has an area of 2 ha with an 18% slope and grassy ground. Ferr3 is about 1.8 ha, 1.3 ha with 0.9% slope and 0.5 ha with 18% slope. The soil is tilled. Of course, all olive groves border on other olive groves with similar characteristics.

In all the selected olive orchards, insecticides were not applied.

The Agrometeorological Service of the Basilicata Region provided the meteorological data for the three study areas.

Sampling of nymphs

The nymphs were sampled once a year in the olive groves with no-tillage. The sampling was carried out in the last ten days of April using the method of quadrats sampling. In 2016 nymphs were collected in 12 samples (1 m² each) randomly distributed in each olive grove and transported to the laboratory alive to be raised to the adult stage. This made it possible to verify our ability to distinguish species on the basis of nymph features reported in the literature (Vilbaste, 1982). In 2017, data refer to 20 samples in each olive grove (as in the previous year) and 40 samples on uncultivated land and road edges in the areas of Bernalda (20 samples) and Metaponto (20 samples). All the spittlebug nymphs were collected, put in ethanol, and transported to the laboratory for identification. The plants on which the spittlebugs were collected were also identified. Plants with no spittlebugs were also recorded. In 2018, sampling was performed as in 2016 but the nymphs were not reared.

Sampling of adults

The samplings of adults were performed monthly within the first ten days of each month, starting from June until October of each year. In September 2018, sampling was performed at the end of the month and samples were not collected in October. A circular sweep net (38 cm diameter) was used to collect insects from 10 randomly selected

sites per grove, except for Met2 and Met3 where 6 sites were sampled each time due to the small area of the grove. In each site, samples were collected in the morning (approximately from 8.00 am to 11.00 am) from olive trees and ground vegetation under the tree canopies or from the peripheral borders of the grove. In each sampling 30 sweeps on ground vegetation and 30 sweeps on tree canopies from four different sides of the trees were performed.

The captured adults were killed in absolute ethanol, transported to the laboratory and determined according to Ossianni (1981). In addition, abdomen of all the specimens captured in the three years (1139 *P. spumarius*; 102 *N. campestris*; 198 *Lepyronia coleoptrata* (L.) (Hemiptera Aphrophoridae); 275 *Cicadella viridis* (L.) (Hemiptera Cicadellidae Cicadellinae) was dissected and inspected for mature eggs and parasitoid larvae. Moreover, to check for the presence of *X. fastidiosa*, the head of all specimens was dissected and subjected to PCR analysis, as described in Saponari *et al.* (2014).

Statistical analysis

We tested for variation of the 4 potential vector species of *X. fastidiosa* among years and vegetation (herbaceous vs olive tree canopies) by applying a two way ANOVA to the number of individuals caught every 30 sweeps after a logarithmic transformation [transformed value = $\ln(1 + \text{original value})$]. Seasonal trends (ln transformed data) of *P. spumarius*, *N. campestris*, *L. coleoptrata* and *C. viridis* catches on herbaceous vegetation (and on olive tree canopy only for *P. spumarius*) were analysed with mixed-model ANOVAs with “year” and “studied area” as main effects and “month” nested within each year.

All the analyses in this study were performed using the R 3.2.3 software (R Core Team, 2014).

Results

Trends of temperature and rainfall

Trends of the medium, maximum and minimum temperatures relating to the three study areas and years (figures S1-S3) and rainfall data (table S1) are available as supplementary material.

The annual temperature trend in 2016-2018 was similar, characterized by a gradual increase in average temperatures from February-March to July-August which

were the warmer months. Compared to the two other years, 2017 had high temperatures in the March-April period, when the development of spittlebug juveniles took place, and a very hot summer with maximum daily temperatures in some cases exceeding 40 °C in August. Over the three years of study rainfall was very different in quantity and, even more, in distribution during the year. Notably, in 2016, the overall rainfall in March-April was 192-200 mm (the lowest value in Bernalda, the highest in Ferrandina), much higher than recorded during the same period in the following years.

In the three study areas, the medium, minimum and maximum temperatures differed by few degrees. Regardless of the years, in Metaponto the average monthly temperature in March was 1 °C higher than recorded in the two other areas. In Ferrandina, despite the average monthly temperature equal to that of Bernalda, the minimum temperatures were higher than in the two other locations. The Bernalda site had the highest summer temperatures.

Potential vector species

P. spumarius, *N. campestris*, *L. coleoptrata* and *C. viridis* were the potential vectors of *X. fastidiosa* captured in the study area. Regarding the three spittlebug species, mature eggs were observed in the abdomen of adult females from the beginning of September.

Larvae of Diptera Pipunculidae (supplemental material figures S4-S5) were found in the abdomen of *N. campestris* adults in June 2017, mainly in the Ferrandina area. The parasitization rates varied with the olive groves, ranging between a minimum of 25% and a maximum of 50%. In the other areas, the parasitoid was substantially absent. A single parasitized *N. campestris* individual was found in Metaponto, none in Bernalda. No parasitoids were found by dissecting the abdomen of *P. spumarius*, *L. coleoptrata* and *C. viridis* adults.

The search for *X. fastidiosa* DNA in the captured insects gave negative results.

Spittlebug nymphs

The average densities of the nymphs of the three spittlebug species are shown in table 1. The list of plant species on which the nymphs were captured is given in table 2. The same table also shows the plant species on which no spittlebugs were found in this study.

Table 1. Number of nymphs per square meter (mean ± standard error); OG = Olive Grove; CA = Compensation Area.

Year	Area	OG/CA	<i>Philaenus spumarius</i>	<i>Neophilaenus campestris</i>	<i>Lepyronia coleoptrata</i>
2016	Metaponto	OG	15.87 ± 2.09	-	-
2016	Bernalda	OG	8.92 ± 1.45	18.17 ± 3.28	-
2016	Ferrandina	OG	17.80 ± 2.78	11.87 ± 4.59	-
2017	Metaponto	OG	3.10 ± 0.94	0.29 ± 0.20	0.10 ± 0.07
2017	Metaponto	CA	3.50 ± 1.20	-	-
2017	Bernalda	OG	6.00 ± 1.06	-	0.17 ± 0.17
2017	Bernalda	CA	5.25 ± 1.66	-	-
2017	Ferrandina	OG	5.51 ± 1.10	2.51 ± 0.81	0.59 ± 0.28
2018	Metaponto	OG	8.86 ± 5.39	0.29 ± 0.28	-
2018	Bernalda	OG	3.57 ± 1.57	1.36 ± 0.82	0.79 ± 0.79
2018	Ferrandina	OG	3.14 ± 1.56	-	-

Table 2. Plant species on which nymphs of *P. spumarius*, *N. campestris* and *L. coleoprata* were collected.

Family	Species	<i>Philaenus spumarius</i>	<i>Neophilaenus campestris</i>	<i>Lepyronia coleoprata</i>
Apiaceae	<i>Daucus carota</i>	+	+	-
Apiaceae	<i>Foeniculum vulgare</i>	+	-	-
Apiaceae	<i>Tordylium apulum</i>	-	-	-
Asteraceae	<i>Anthemis arvensis</i>	+	+	-
Asteraceae	<i>Calendula arvensis</i>	+	-	-
Asteraceae	<i>Cichorium intybus</i>	+	-	-
Asteraceae	<i>Cirsium arvensis</i>	+	-	-
Asteraceae	<i>Cirsium</i> sp.	+	-	-
Asteraceae	<i>Crepis biennis</i>	+	-	-
Asteraceae	<i>Crepis neglecta</i>	+	-	-
Asteraceae	<i>Crepis pulchra</i>	-	-	-
Asteraceae	<i>Crepis</i> sp.	+	-	-
Asteraceae	<i>Glebionis coronaria</i>	+	-	-
Asteraceae	<i>Hedypnois rhagadioloides</i>	+	-	-
Asteraceae	<i>Dittrichia viscosa</i>	+	-	-
Asteraceae	<i>Pallenis spinosa</i>	+	-	-
Asteraceae	<i>Picris hieracioides</i>	+	-	-
Asteraceae	<i>Reichardia picroides</i>	-	-	-
Asteraceae	<i>Scorzonera aristata</i>	+	-	-
Asteraceae	<i>Sonchus arvensis</i>	+	-	+
Asteraceae	<i>Sonchus asper</i>	+	-	-
Asteraceae	<i>Sonchus oleraceus</i>	+	-	-
Asteraceae	<i>Tolpis umbellata</i>	+	-	-
Asteraceae	<i>Tragopogon porrifolius</i>	-	-	-
Asteraceae	<i>Urospermum dalechampii</i>	+	-	-
Asteraceae	<i>Urospermum picroides</i>	+	-	-
Brassicaceae	<i>Lepidium draba</i>	+	-	-
Brassicaceae	<i>Sinapis arvensis</i>	-	-	-
Caprifoliaceae	<i>Sixalix atropurpurea</i>	+	-	-
Caryophyllaceae	<i>Silene</i> sp.	+	-	-
Convolvulaceae	<i>Convolvulus arvensis</i>	-	-	-
Convolvulaceae	<i>Convolvulus cantabrica</i>	-	-	-
Fabaceae	<i>Coronilla scorpioides</i>	+	-	-
Fabaceae	<i>Hymenocarpus circinnatus</i>	-	-	-
Fabaceae	<i>Lathyrus nissolia</i>	-	-	-
Fabaceae	<i>Lathyrus ochrus</i>	+	-	-
Fabaceae	<i>Lotus angustissimus</i>	+	+	-
Fabaceae	<i>Medicago blanchiana</i>	-	-	-
Fabaceae	<i>Medicago ciliaris</i>	+	-	-
Fabaceae	<i>Medicago rigidula</i>	+	-	-
Fabaceae	<i>Medicago scutellata</i>	+	-	-
Fabaceae	<i>Medicago</i> sp.	+	-	+
Fabaceae	<i>Scorpiurus muricatus</i>	-	-	-
Fabaceae	<i>Trifolium alexandrinum</i>	+	-	-
Fabaceae	<i>Trifolium stellatum</i>	-	-	-
Fabaceae	<i>Vicia melanops</i>	+	-	-
Fabaceae	<i>Vicia pseudocracca</i>	+	-	-
Fabaceae	<i>Vicia</i> sp.	+	-	-
Geraniaceae	<i>Erodium cicutarium</i>	+	-	-
Geraniaceae	<i>Erodium malacoides</i>	+	-	-
Geraniaceae	<i>Geranium</i> sp.	-	-	-
Liliaceae	<i>Muscari comosum</i>	+	-	-
Malvaceae	<i>Malva sylvestris</i>	+	-	-
Orchidaceae	<i>Serapias vomeracea</i>	-	-	-
Orchidaceae	<i>Sherardia arvensis</i>	+	-	-
Orobanchaceae	<i>Orobanche</i> sp.	-	-	-
Plantaginaceae	<i>Plantago lagopus</i>	+	-	-
Poaceae	<i>Avena sterilis</i>	+	+	-
Poaceae	<i>Briza maxima</i>	-	-	-
Poaceae	<i>Bromus mollis</i>	+	+	-
Poaceae	<i>Bromus sterilis</i>	+	+	-
Poaceae	<i>Dactylis glomerata</i>	-	-	-
Poaceae	<i>Dasyphyrum villosum</i>	+	+	-
Primulaceae	<i>Anagallis arvensis</i>	+	-	-
Primulaceae	<i>Anagallis parviflora</i>	+	-	-

The range of plants on which *P. spumarius* nymphs were collected was much broader than that of *N. campestris* and *L. coleoptrata* (table 2). The average number of *P. spumarius* nymphs per plant, in the case of different host plant species, is shown in table 3. *Urospermum dalechampii* (L.), *Crepis biennis* L., *Sixalix atropurpurea* (L.), *Glebionis coronaria* (L.) and *Avena sterilis* L. were the species with the highest aggregation of *P. spumarius*. Most of the host plant species were present both in the olive groves and in the compensation areas. *Glebionis coronaria*, that abundantly hosted the juvenile stages of *P. spumarius*, was mainly present in the compensation areas or on the peripheral borders of the olive groves.

In the compensation areas, most of the *P. spumarius* nymphs were collected on Asteraceae (59%), with greater aggregation on *G. coronaria*. This last plant species hosted 22-27% of the total *P. spumarius* nymphs, depending on the area (Bernalda or Metaponto). Also, in the compensation areas, 20-21% of the *P. spumarius* nymphs were on Fabaceae, mainly *Medicago* spp. In the olive groves in Metaponto, the Fabaceae hosted most of the *P. spumarius* nymphs (72%), mainly on *Medicago* (32%) and *Vicia* (23%). In the two other areas, under the olive trees, 30-31% of the nymphs of *P. spumarius* were collected on Asteraceae and 34-39% on Fabaceae.

Table 3. *P. spumarius*: aggregation of nymphs on different plant species (average number of individuals per plant).

Family	Species	mean	S.E	N
Asteraceae	<i>Urospermum dalechampii</i>	17.7	14.1	6
Asteraceae	<i>Crepis biennis</i>	11.0	3.0	2
Caprifoliaceae	<i>Sixalix atropurpurea</i>	10.0	-	1
Asteraceae	<i>Glebionis coronaria</i>	7.0	2.0	6
Poaceae	<i>Avena sterilis</i>	6.8	1.7	10
Poaceae	<i>Dasyphyrum villosum</i>	5.5	3.5	2
Poaceae	<i>Bromus sterilis</i>	5.0	2.0	4
Fabaceae	<i>Trifolium alexandrinum</i>	5.0	-	1
Asteraceae	<i>Anthemis arvensis</i>	4.8	2.6	4
Fabaceae	<i>Medicago</i> sp.	4.2	0.7	30
Asteraceae	<i>Sonchus arvensis</i>	4.2	1.5	5
Malvaceae	<i>Malva sylvestris</i>	4.0	-	1
Asteraceae	<i>Cichorium intybus</i>	3.7	1.3	3
Orchidaceae	<i>Sherardia arvensis</i>	3.3	1.5	3
Apiaceae	<i>Daucus carota</i>	3.2	1.2	6
Fabaceae	<i>Vicia</i> sp.	3.2	0.8	13
Geraniaceae	<i>Erodium cicutarium</i>	3.0	1.0	3
Fabaceae	<i>Lotus angustissimus</i>	3.0	0.0	2
Liliaceae	<i>Muscari comosum</i>	3.0	-	1
Asteraceae	<i>Sonchus oleraceus</i>	2.6	0.7	5
Asteraceae	<i>Picris hieracioides</i>	2.5	0.5	2
Asteraceae	<i>Calendula arvensis</i>	2.0	1.0	2
Asteraceae	<i>Cirsium</i> sp.	2.0	-	1
Asteraceae	<i>Ditrichia viscosa</i>	2.0	0.6	3
Asteraceae	<i>Sonchus asper</i>	2.0	0.0	2
Asteraceae	<i>Pallenis spinosa</i>	2.0	-	1
Asteraceae	<i>Scorzonera aristata</i>	2.0	-	1
Brassicaceae	<i>Lepidium draba</i>	2.0	-	1
Poaceae	<i>Lolium perenne</i>	2.0	0.4	5
Poaceae	<i>Bromus mollis</i>	1.7	0.3	3
Plantaginaceae	<i>Plantago lagopus</i>	1.5	0.5	2
Asteraceae	<i>Crepis neglecta</i>	1.2	0.2	5
Apiaceae	<i>Foeniculum vulgare</i>	1.0	-	1
Asteraceae	<i>Cirsium arvensis</i>	1.0	-	1
Asteraceae	<i>Cirsium asper</i>	1.0	-	1
Asteraceae	<i>Hedypnois rhagadioloides</i>	1.0	-	1
Asteraceae	<i>Tolpis umbellata</i>	1.0	-	1
Asteraceae	<i>Urospermum picroides</i>	1.0	-	1
Caryophyllaceae	<i>Silene</i> sp.	1.0	-	1
Fabaceae	<i>Coronilla scorpioides</i>	1.0	0.0	2
Fabaceae	<i>Lathyrus ochrus</i>	1.0	-	1
Geraniaceae	<i>Erodium malacoides</i>	1.0	0.0	3
Primulaceae	<i>Anagallis arvensis</i>	1.0	-	1
Primulaceae	<i>Anagallis parviflora</i>	1.0	0.0	2

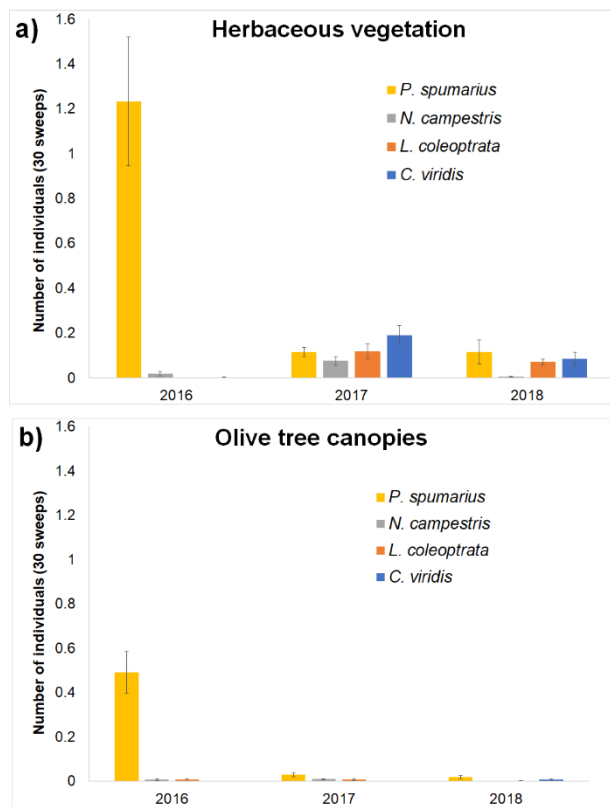


Figure 1. Number of individuals (mean \pm standard error) of the 4 potential vector species of *X. fastidiosa*, caught every 30 sweeps during the year on the herbaceous vegetation (a) and on olive tree canopies (b).

Trends of adult catches

Among the four potential vectors of *X. fastidiosa* found in the study area, *P. spumarius* was by far the most dominant in 2016. In 2017 and 2018 the catch rates were moderate for all species (figure 1).

Differences among years were highly significant for all species (*P. spumarius*: $F_{2,1602} = 92.07$, $P < 0.001$; *N. campestris*: $F_{2,1602} = 12.07$, $P < 0.001$; *L. coleoprata*: $F_{2,1602} = 7.53$, $P < 0.001$; *C. viridis*: $F_{2,1602} = 10.18$, $P < 0.001$). Catches were always much more abundant on ground vegetation than on trees (*P. spumarius*: $F_{1,1602} = 18.35$, $P < 0.001$; *N. campestris*: $F_{1,1602} = 18.33$, $P < 0.001$; *L. coleoprata*: $F_{1,1602} = 46.76$, $P < 0.001$; *C. viridis*: $F_{1,1602} = 46.76$, $P < 0.001$) (figure 1). The catches of *P. spumarius* on ground vegetation or trees showed the same trend over the years since the interaction between these two factors was not significant ($F_{2,1602} = 2.46$, $P = 0.086$). For the three other species, the interaction between catches on the ground/canopy and the year was instead significant (*N. campestris*: $F_{2,1602} = 7.04$, $P < 0.001$; *L. coleoprata*: $F_{2,1602} = 8.17$, $P < 0.001$; *C. viridis*: $F_{2,1602} = 10.78$, $P < 0.001$). In these cases, in the years characterized by higher catches, only the number of individuals captured on the ground vegetation increased. In contrast, the number on the olive trees remained more or less constant.

Trends of *P. spumarius* catches in the three study areas are shown in figure 2 (ground vegetation) and figure 3

(olive canopies). The catch rates of *P. spumarius* on herbaceous vegetation significantly changed with the studied areas ($F_{6,757} = 21.78$, $P < 0.001$) and with the months within each year ($F_{11,757} = 80.45$, $P < 0.001$). The month within year-by-area interaction was also significant ($F_{21,757} = 16.2$, $P < 0.001$). A conspicuous peak of catches on herbaceous vegetation was observed in June 2016 in the Ferrandina and Metaponto areas. Afterwards, the average number of captured individuals collapsed to zero in July and August. It recovered slightly in September-October but the density was anyway very low. In the following two years, the June peak was inconspicuous: one or less than one individual per 30 sweeps (figure 2).

The catch rates of *P. spumarius* on olive canopies also significantly changed with the study areas ($F_{6,769} = 18.8$, $P < 0.001$) and with the months within each year ($F_{11,769} = 34.75$, $P < 0.001$). Also, in this case, the month within

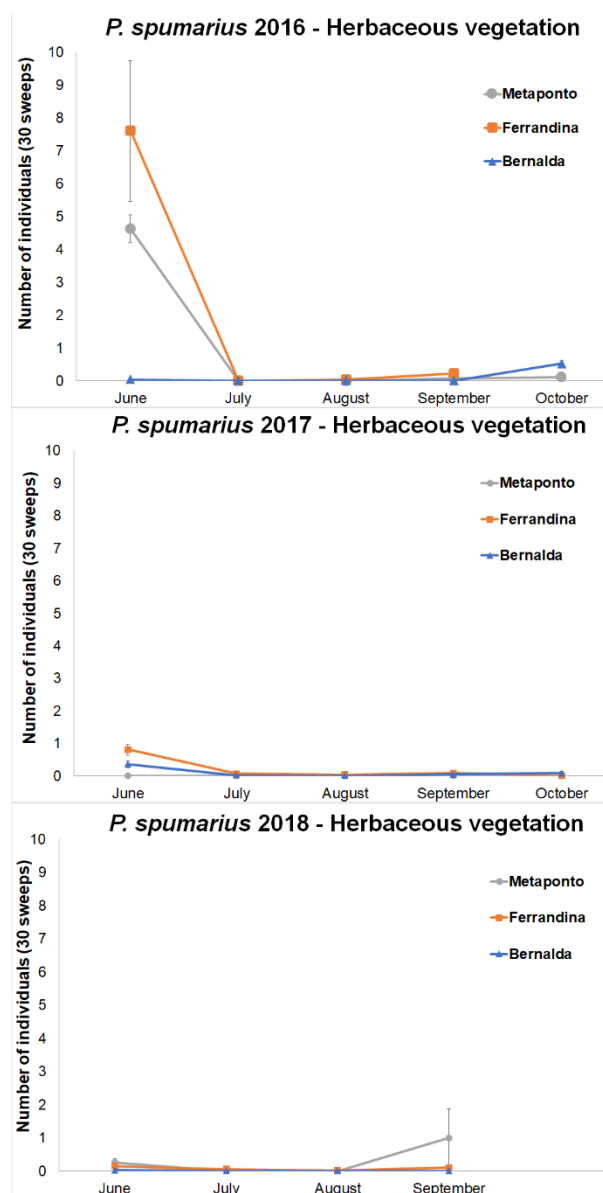


Figure 2. *P. spumarius*: trends of catches (average number of individuals caught by 30 sweeps) on the ground vegetation, year by year, in the studied areas.

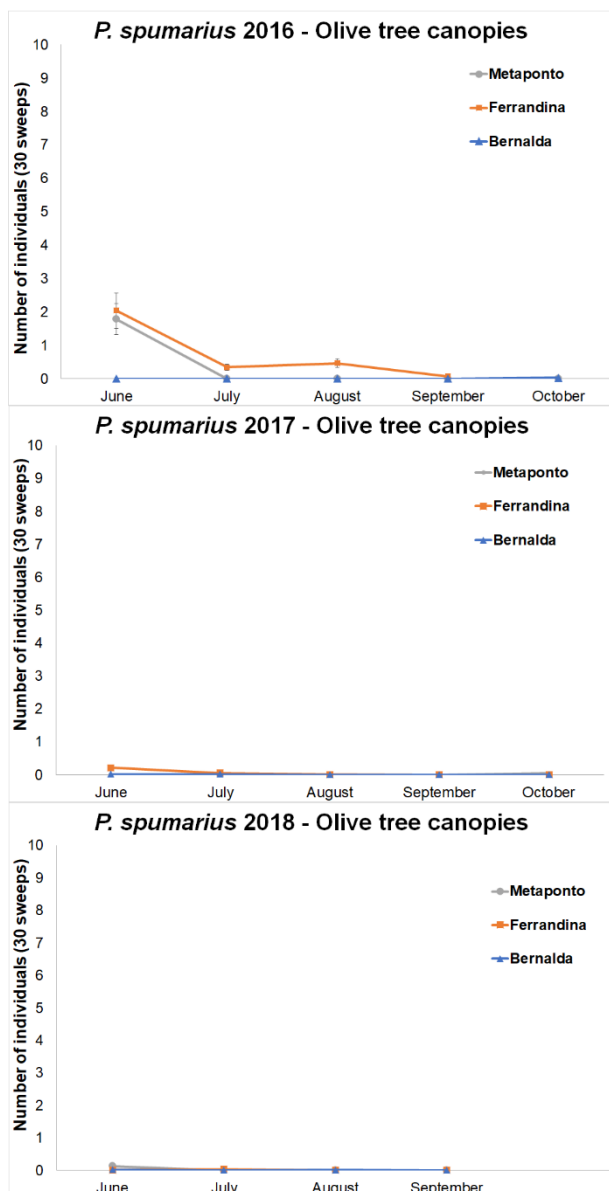


Figure 3. *P. spumarius*: trends of catches (average number of individuals caught by 30 sweeps) on tree canopies, year by year, in the studied areas.

year-by-area interaction was significant ($F_{21,769} = 5.95$, $P < 0.001$). In the areas where the catch rates of *P. spumarius* on ground vegetation were high in June 2016, the spittlebugs were also captured on the crown of the olive trees. In the following months *P. spumarius* was captured exclusively on the ground vegetation. In Ferrandina, *P. spumarius* was caught on olive trees in July and August, but with a lower catch rate than in June. Catches on olive trees were zero or near zero in 2017 and 2018.

In the case of *N. campestris*, *L. coleoptrata* and *C. viridis*, we only analysed the catch trends on the herbaceous vegetation, since the catches on olive trees were rare (less than 0.01 individuals per 30 sweeps).

N. campestris catches in the different areas ($F_{6,757} = 5.44$, $P < 0.001$), in the different periods within year ($F_{11,757} = 16.58$, $P < 0.001$) and the interaction month within year-by-area ($F_{21,757} = 3.78$, $P < 0.001$) were highly significant. *N. campestris* was not, or very rarely

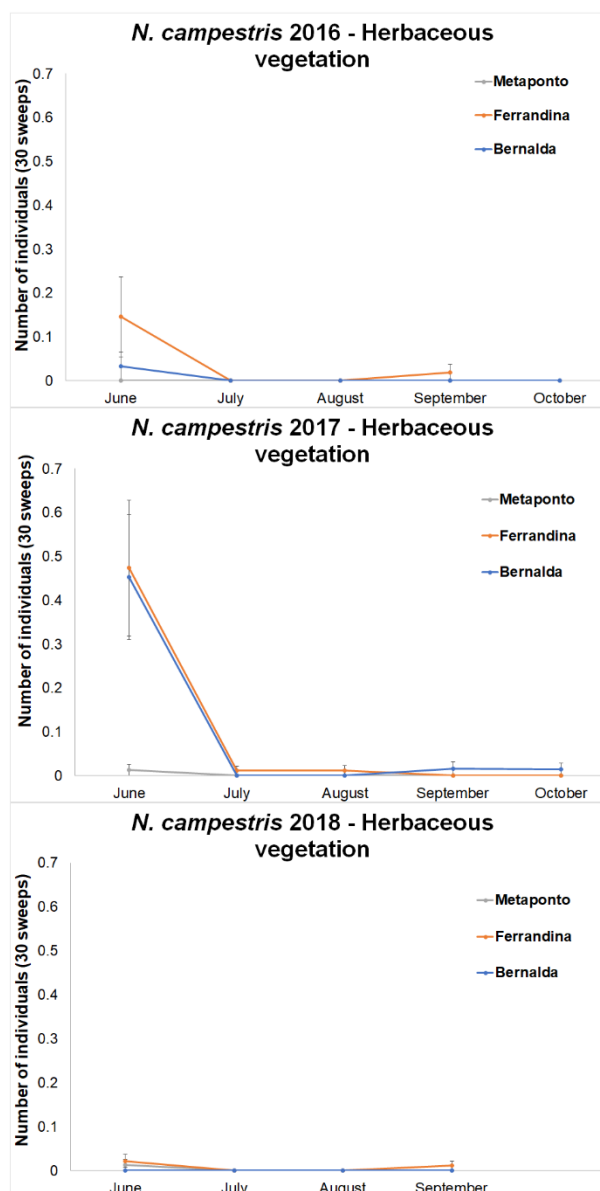


Figure 4. *N. campestris*: trends of catches (average number of individuals caught by 30 sweeps) on the ground vegetation, year by year, in the studied areas.

present in the Metaponto area (figure 4). This species was instead captured, with different rates according to the years, in Bernalda and Ferrandina areas, especially in June (figure 4).

The trend of *L. coleoptrata* catches is shown in figure 5. Catches of this species in the different areas ($F_{6,757} = 2.42$, $P = 0.025$) and in the different periods ($F_{11,757} = 3.77$, $P < 0.001$) within the year were significant, while the interaction month within year-by-area was not significant ($F_{21,757} = 0.74$, $P = 0.79$). *L. coleoptrata* was not captured in 2016. Instead, in 2017 and 2018, this species was captured in all the three study areas. The catch trends were different in the two years. In 2017 catch rates gradually decreased during the summer, faster in Bernalda than in Ferrandina. In 2018 catch rate, although fluctuating, did not show a clear downward trend.

Figure 6 shows the average number of *C. viridis* individuals caught by 30 sweeps in the different areas according

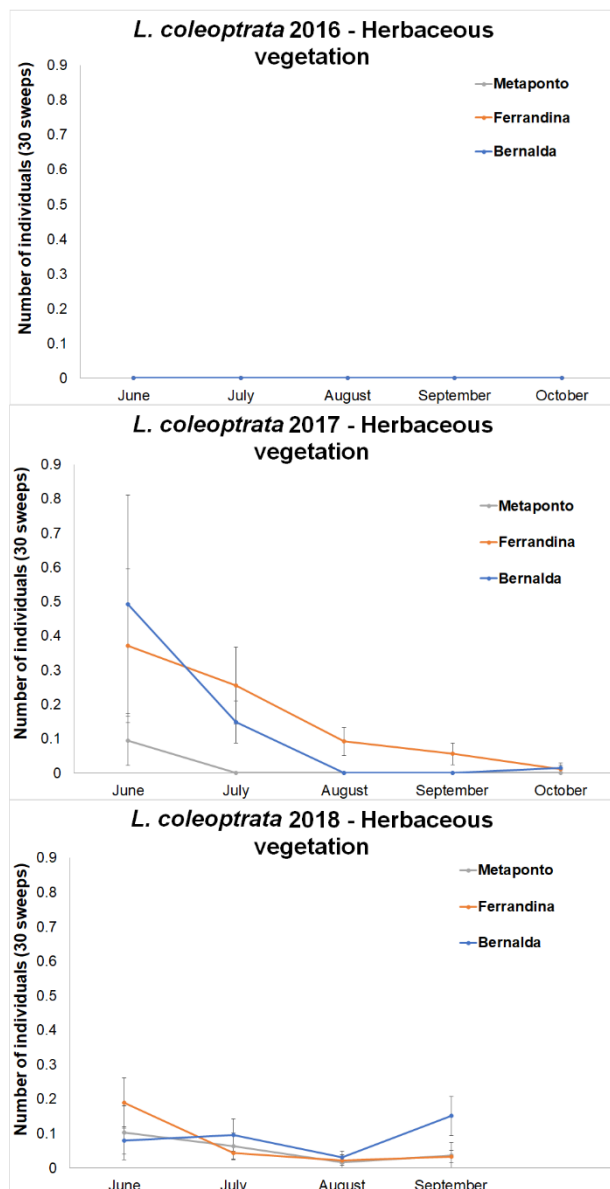


Figure 5. *L. coleoptrata*: trends of catches (average number of individuals caught by 30 sweeps) on the ground vegetation, year by year, in the studied areas.

to the months and years. In the case of *C. viridis*, the number of individuals caught includes both adults and juvenile stages. In 2016, only a few individuals were caught in Bernalda in October. In 2017, rather low catches were observed in Metaponto and more in Bernalda and Ferrandina. In these two areas the highest catch rate was recorded in July. However, while catches decreased in Bernalda since August, they remained high in Ferrandina throughout the summer. In 2018, catches in Ferrandina remained low throughout the sampling period. In 2018, in Metaponto area, the number of *C. viridis* peaked in July, while its number showed a growing trend until the end of September in Bernalda. Over the years, the catch differences in the study areas were highly significant ($F_{6,757} = 4.54$, $P < 0.001$). On the contrary, neither the differences among months were significant ($F_{11,757} = 1.78$, $P < 0.053$), nor the interaction month within year-

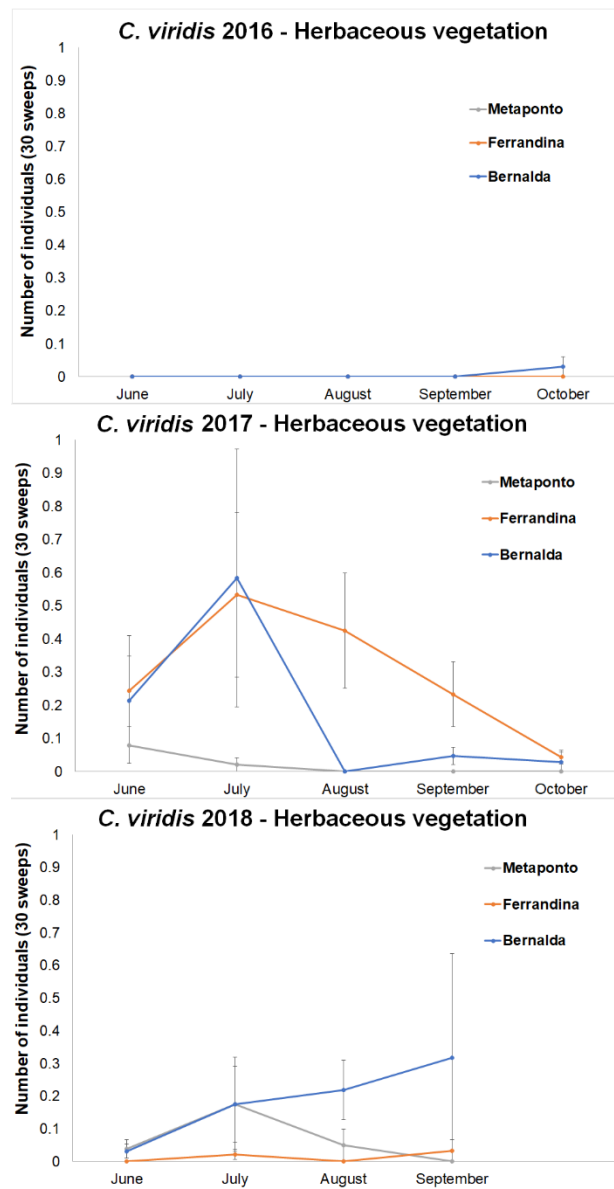


Figure 6. *C. viridis*: trends of catches (average number of individuals caught by 30 sweeps) on the ground vegetation, year by year, in the studied areas.

by-area ($F_{21,757} = 0.66$, $P = 0.87$). Catches of *C. viridis* have always been very localized in those spots of the olive groves, where the shape of the soil favours water accumulation.

Discussion

This survey of the potential vectors of *X. fastidiosa* in olive orchards, located in three areas of the Basilicata Region, showed the presence of three Aphrophoridae species: *P. spumarius*, *N. campestris* and *L. coleoptrata*. *P. spumarius* and *N. campestris* are two widespread species in Italy and other southern European Countries (Lopes *et al.*, 2014; Morente *et al.*, 2018; Tsagkarakis *et al.*, 2018; Bodino *et al.*, 2019; Theodorou *et al.*, 2021) while *L. coleoptrata*, not reported for *X. fastidiosa*-

infected olive groves of the Apulia region, was occasionally captured in olive groves of northern Italy (Liguria) (Bodino *et al.*, 2019), south Greece (Chania, Crete) (Theodorou *et al.*, 2021) and central Spain (Morata de Tajuña, near Madrid) (Morente *et al.*, 2018). Some other putative vectors of *X. fastidiosa*, captured in olive groves of Italy, Greece and Spain, even sporadically, were not detected during the three-year study in Basilicata. It is the case of *Aphrophora alni* (Fallen) (Antonatos *et al.*, 2019; Bodino *et al.*, 2019; Theodorou *et al.*, 2021), *P. italosignus* (Panzavolta *et al.*, 2019; Theodorou *et al.*, 2021), *Neophilaenus lineatus* (L.) (Theodorou *et al.*, 2021), *C. sanguinolenta* (Scopoli) (Theodorou *et al.*, 2021), *Cercopis intermedia* Kirschbaum (Morente *et al.*, 2018). Among these species, for the time being, only *P. italosignus* proved to be a competent vector of *X. fastidiosa* subsp. *pauca* ST53, associated with the OQDS in Italy (Cavaliere *et al.*, 2019). Furthermore, the cicadellid *C. viridis* was frequently captured in the olive groves of Basilicata but was not reported for other olive growing areas in Italy and southern Europe. Weed species composition is known to affect Auchenorrhyncha fauna. It could account, at least partially, for the differences observed in the abundance and richness of putative *X. fastidiosa* vector species in the European olive growing areas. Notably, *P. italosignus* is conditioned by the presence of *Asphodelus ramosus* L. (Panzavolta *et al.*, 2019), which is absent in the study areas in Basilicata.

Besides plant composition, other factors can influence the dominance in Auchenorrhyncha communities. For instance, *P. spumarius* was a dominant species in Basilicata only in 2016, despite the abundance of the favoured host plants for the development of the juvenile stages. The fluctuation of *P. spumarius* populations, observed over the years, was likely caused mainly by climatic factors. Climatic factors, and especially spring and summer drought, are known to influence the distribution (Karban and Strauss, 2004) and population density (Drosopoulos and Asche, 1991) of *P. spumarius*. In favourable years, *P. spumarius* becomes the dominant species probably thanks to the availability of many plant species on which the juvenile stages can feed.

In England, *P. spumarius* adults can be parasitized by the pipunculid fly *Verrallia aucta* Fallen (Harper and Whittaker, 1976). The presence of *V. aucta* was recently confirmed for Northern Italy, where the percentage of parasitized *P. spumarius* adults rarely exceeded 15% (Molinatto *et al.*, 2020). During this three-year study, no individuals of *P. spumarius* parasitized by pipunculid dipterans were found, which could instead be an important containment factor for *N. campestris* in Basilicata. We do not know if the parasitoid we detected in *N. campestris* is ascribable to the species *V. aucta*. Molinatto *et al.* (2020) provided evidence that *V. aucta* is not species-specific, and it can parasitize *N. campestris*, although with low prevalence. However, it is probable that, in our case, the dipterous larvae belong to a different species, as already reported by Whittaker (1969).

The catch rate of *P. spumarius* on the crown of olive trees has always been lower than on the ground vegetation, which is consistent with what other authors have observed (Morente *et al.*, 2018; Thanou *et al.*, 2021). We

cannot exclude that this outcome is the consequence of the sampling method used. The sweep net is known to be a poorly effective tool for sampling insects from a tree canopy compared to its high efficacy on the ground cover (Purcell *et al.*, 1994; Morente *et al.*, 2018), even if sweep net is still the most efficient method compared the available ones (Morente *et al.*, 2018). Furthermore, the daytime at which the sampling is carried out might also have an influence if the insects move from the herbaceous vegetation to the tree canopy and vice versa during the day. The highest catch rates on the olive trees were recorded in early June 2016 when the highest abundance of *P. spumarius* adults in the olive groves was also recorded, and there was still a wide availability of herbaceous plants in full vegetation. Starting from July, the populations of *P. spumarius* in the olive groves collapsed, due to the well-known migratory tendency of this species adults (Drosopoulos *et al.*, 2010; Bodino *et al.*, 2020). No shift or permanence of *P. spumarius* populations on the olive trees was observed as described for the olive groves in Liguria and the coastal area of the Apulia region (Bodino *et al.*, 2019). The trend is more like the one described for the internal area of the Apulia region (Bodino *et al.*, 2019) and mainland Spain and northeastern Portugal (Morente *et al.*, 2018). There is some evidence that landscape composition of the close surrounding of the olive groves may affect *P. spumarius* spatial distribution (Santoiemma *et al.*, 2019; Bodino *et al.*, 2020). It is possible that, in the presence of a variety of crops and spontaneous plants, *P. spumarius* adults tend to leave the olive groves, attracted to other hosts in the summer. Moreover, little is known about the influence of olive genotype, vegetative, nutritional and sanitary state of the foliage on olive suitability and attractiveness for *P. spumarius*.

In the study areas *N. campestris* was not abundant and had a seasonal catch trend very similar to that observed for *P. spumarius*. On the contrary, *L. coleoptrata* tended to persist in the olive groves during the summer, placing itself on perennial herbaceous plants. Both *N. campestris* and *L. coleoptrata* were sporadically captured on olive canopies. The life cycle of *L. coleoptrata* had been formerly studied in northeastern Italy, where this species has three annual generations and nymphs can be collected from May to November (Barro and Pavan, 1999). In Basilicata, *L. coleoptrata* undergoes an ovarian diapause like *P. spumarius*. Indeed, no nymphs were observed from June and, when dissecting adult females, mature eggs were found in their abdomen only from the beginning of September. This may be an adaptation to the dry climate that characterizes late spring and summer in southern Italy.

C. viridis was captured in all the three study areas but its presence within the olive groves was confined to the patches where the conformation of the soil favours water accumulation and the development of suitable habitat for this species. The trend of catches was very different between the areas over the years. It can be influenced by various factors, such as the activity of parasitoids and predators and the active dispersion of adults (Beok, 1972). The movements of *C. viridis* are essentially confined within the habitat. It seems to be confirmed since we have always caught this species in the same sites.

However, *C. viridis* may be a potential migrant, which could explain its presence in habitats that are of a discontinuous nature (Beok, 1972).

The subfamily Cicadellinae includes the most important known vectors of *X. fastidiosa* in the Americas (Redak *et al.*, 2004). Nevertheless, it is unlikely that *C. viridis* may play a role in transmitting CoDiRO strain of *X. fastidiosa* to olive, also because this sharpshooter was hardly captured on olive canopies.

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