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BEYOND XYLELLA

Best agronomic practices for the management of tolerant/resistant cultivars in infected areas

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SUMMARY

The use of resistant and tolerant olive cultivars is a central strategy for restoring olive production in areas affected by *Xylella fastidiosa* subsp. *pauca* in Apulia, southern Italy. However, their long-term performance under natural inoculum pressure depends on vector populations, agronomic management and local epidemiological conditions. Within BeXyl Task 7.2, this study evaluated the phytosanitary status and infection dynamics of newly established olive groves planted with resistant cultivars, with the aim of identifying factors relevant to their sustainable management in infected areas.

The study was conducted in 16 commercial olive groves planted with 'Leccino' and 'Fs17' in the infected area of Apulia. The groves represented different agronomic conditions, including conventional soil tillage or no tillage with mulching, and different planting densities according to cultivar. From 2023 to 2025, populations of the main spittlebug vectors, *Philaenus spumarius* and *Neophilaenus campestris*, were monitored through surveys of juveniles on ground-cover vegetation and of adults using yellow sticky traps in olive canopies and sweep-net sampling. Tree infection status was assessed by qPCR in 2024 and 2025, while six infected trees per grove were selected to investigate within-canopy infection dynamics.

Juvenile populations were dominated by *P. spumarius*, with higher densities generally observed in mulched groves and along unmanaged field borders. Adult vectors showed clear seasonal dynamics, with higher abundance early in the season and higher densities on ground-cover vegetation in mulched than in tilled groves. Field-level *X. fastidiosa* incidence ranged from 1.68% to 69.45% and increased from 2024 to 2025 in all monitored groves. No significant difference in infection incidence was detected between the two soil-management strategies, whereas cultivar and vector-density class were significantly associated with infection levels. Overall, estimated infection incidence was lower in 'Leccino' than in 'Fs17' groves, and fields classified as having low vector density showed lower infection incidence than those with medium or high vector density. Despite qPCR-confirmed infections, symptoms were absent or limited in most fields. Within-canopy analyses revealed a heterogeneous and temporally dynamic distribution of *X. fastidiosa* within infected trees.

Overall, resistant cultivars are essential for restoring olive production in infected areas, but they are not sufficient as a stand-alone measure to prevent pathogen spread. Their long-term success depends on integrated, site-specific management combining cultivar resistance, vector suppression, reduction of local inoculum pressure, and long-term epidemiological monitoring.

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1 INTRODUCTION

This deliverable summarizes the results obtained within WP7 Task 7.2 on the management of resistant olive cultivars in areas with established *X. fastidiosa* epidemics. The study assessed the effects of different agronomic management practices and vector densities on the spread and persistence of infections in resistant olive groves, providing insights into the durability of resistance and the resilience of these production systems.

The work took advantage of the Apulian regional regeneration programme, which supported the establishment of new olive groves with resistant cultivars in infected areas. A network of 3–4-year-old commercial olive groves was monitored to evaluate infection prevalence and vector abundance under different epidemiological and management scenarios. This approach made it possible to collect meaningful data within the project timeframe, which would not have been feasible using newly established experimental plantations because of the long incubation period of *X. fastidiosa* infections.

Furthermore, the use of commercial groves enabled the assessment of management practices at a large spatial scale and under real farming conditions, overcoming the limitations of small experimental plots and better reflecting the variability of commercial orchard management.

2 MATERIAL AND METHODS

2.1 Selection of the olive groves and design of the experimental parcels

In early 2023, Partners P17-CRSFA and P24-UNAPROL identified a network of 20 commercial olive groves, each covering approximately 1 ha, located within the demarcated infected area of Apulia. Following an assessment of management practices and site accessibility, 16 groves were selected and monitored throughout the project (Milestone M19).

The selected groves consisted of 3–4-year-old olive trees of the *X. fastidiosa*-resistant cultivars ‘Fs17’ and ‘Leccino’. The groves differed in terms of soil management, planting density, canopy management, and surrounding landscape characteristics. The following agronomic and environmental factors were considered:

1) Soil management:
    - Tillage;
    - No tillage with mulching.
2) Plant density and canopy management:
    - For ‘Leccino’:
        - Low density (LD): 200-300 trees ha^{-1};
        - High density (HD): 400-500 trees ha^{-1}.
    - For ‘Fs17’:
        - High density (HD): 400-500 trees ha^{-1};
        - Super high density (SHD): 800-1100 trees ha^{-1}.
3) Surrounding landscape:
    - Presence of highly symptomatic centennial olive orchards;
    - Mediterranean pasture vegetation;

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- Other evergreen shrubs and landscape trees.

The location and main characteristics of the selected groves are reported in **Table 1** and illustrated in **Figure 1**.

All selected groves were equipped with irrigation systems because no recently established non-irrigated olive orchards meeting the experimental requirements were available within the infected area of Apulia. Consequently, irrigation could not be included as an experimental factor in the study.

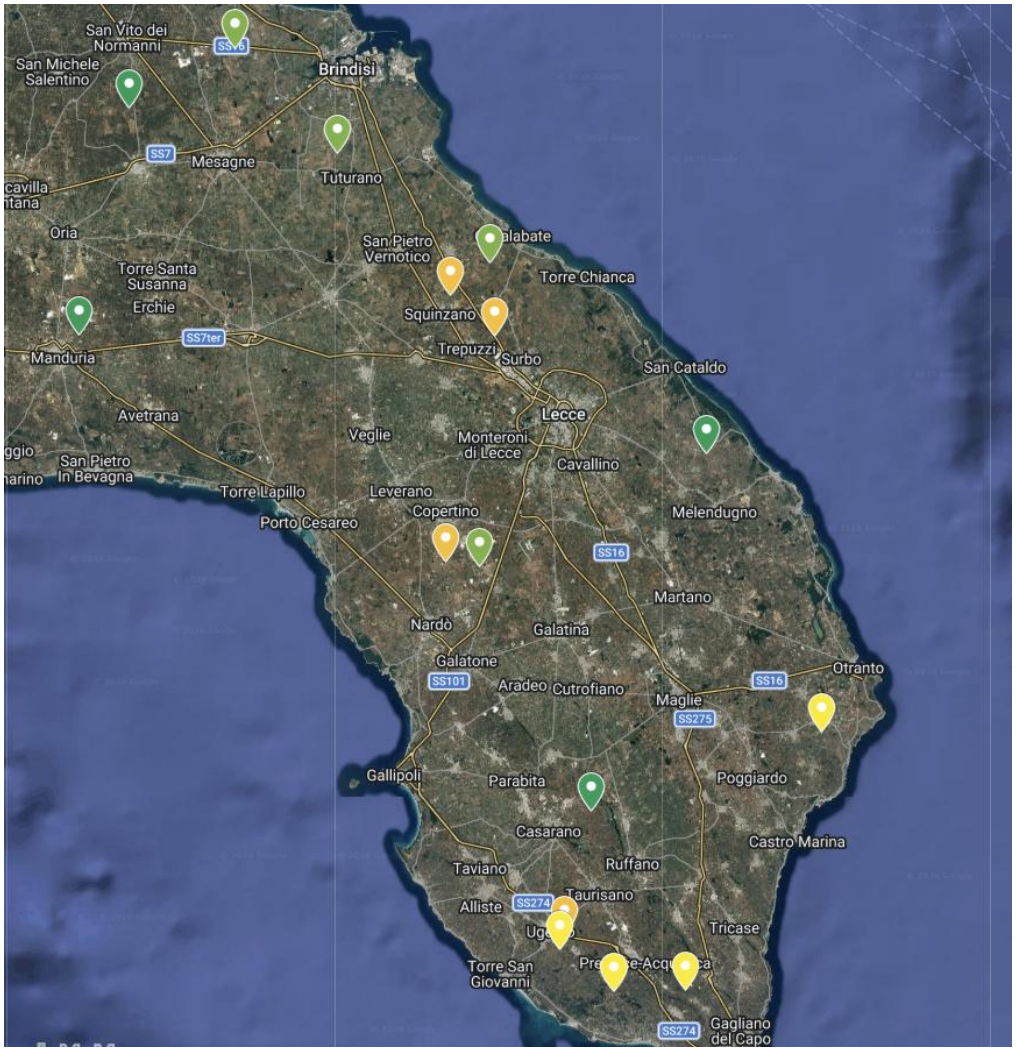
Similarly, newly established almond and cherry orchards were not included because no commercial orchards with suitable size, age, and management characteristics were available to support a scientifically robust comparison. Therefore, the study focused exclusively on olive groves established under the regional regeneration programme.

Table 1. Locations of the selected olive groves.

Plot ID	Country	Town/Village (Province)	GPS Coordinates		Cultivar	Plant density	N. trees monitored in each field
1	Italy	Latiano (BR)	40°36'5.88"N	17°42'22.24"E	'Leccino'	LD	288
2	Italy	Manduria (TA)	40°25'0.76"N	17°39'13.43"E	'Leccino'	LD	211
3	Italy	Casarano (LE)	40°1'34.838''N	18°12'8.762''E	'Leccino'	LD	210
4	Italy	Vernole (LE)	40°19'08.7''N	18°19'49.8''E	'Leccino'	LD	221
5	Italy	Brindisi (BR)	40°39'0.51"N	17°49'18.22"E	'Leccino'	HD	476
6	Italy	Brindisi (BR)	40°33'56.99"N	17°55'58.20"E	'Leccino'	HD	352
7	Italy	Squinzano (BR)	40°28'30.4''N	18°05'39.4''E	'Leccino'	HD	544
8	Italy	Galatina (LE)	40°13'39.62"N	18° 4'59.36"E	'Leccino'	HD	418
9	Italy	Trepuzzi (BR)	40°24'47.65''N	18°5'39.127''E	'Fs17'	SHD	825
10	Italy	Squinzano (LE)	40°26'58.58"N	18° 3'8.03"E	'Fs17'	SHD	923
11	Italy	Nardò (LE)	40°13'38.01"N	18° 2'40.51"E	'Fs17'	SHD	768
12	Italy	Ugento (LE)	39°55'31.6''N	18°10'24.1''E	'Fs17'	SHD	1020
13	Italy	Otranto (LE)	40° 5'29.76"N	18°26'59.80"E	'Fs17'	HD	432
14	Italy	Alessano (LE)	39°52'43.74"N	18°18'18.76"E	'Fs17'	HD	343
15	Italy	Ugento (LE)	39°54'43.91"N	18°10'6.06"E	'Fs17'	HD	173
16	Italy	Acquarica (LE)	39°52'43.28"N	18°13'40.05"E	'Fs17'	HD	144

BR = Brindisi; Le= Lecce; TA= Taranto

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Legend:

- sites with high density olive grove cv 'Leccino'
- sites with low density olive grove cv 'Leccino'
- sites with high density olive grove cv 'Fs17'
- sites with super high-density olive grove cv 'Fs17'

Figure 1. Map showing the distribution of the experimental sites in Apulia (Italy) monitored during the project.

2.2 Monitoring approaches for insect vectors and trees

Starting in 2023, a systematic monitoring programme was carried out annually in each olive grove over three consecutive years to assess:

a) The population density of juvenile and adult stages of *P. spumarius* and *N. campestris*;
b) The presence of symptoms compatible with *X. fastidiosa* infection through visual inspections;
c) The infection status of olive trees by *X. fastidiosa*, including a sampling for monitoring the within-plant progression of the infections (sampling multiple branches).

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2.2.1 Population density of juveniles and adults of *P. spumarius* and *N. campestris*

During spring, the juvenile populations were assessed through a single survey conducted when most individuals were expected to be at the peak of the third and fourth nymphal instars. Twenty randomly distributed sampling units were inspected within each selected grove. In addition, 40 sampling units were assessed along the grove borders (10 per side), where no soil-management practices were applied.

Each sampling unit consisted of a 0.25 m² quadrat, in which the number of spittle masses and nymphs was recorded. Nymphs were identified to species level, distinguishing *P. spumarius* from *N. campestris*.

Adult populations were monitored using yellow sticky traps installed in the olive canopy and sweep-net sampling on ground-cover vegetation. Sticky traps were positioned both within the central area of each grove and along its external borders. The number of traps depended on grove size, tree number, and plot perimeter, with approximately one trap installed every 25–30 linear metres. The number of traps monitored in each grove is reported in Table 2.

Adult populations on ground-cover vegetation were estimated by sweep-net sampling, performing 30 randomly distributed sweeps per grove on each sampling date.

Monitoring was carried out every two weeks during 2023 and 2024, and monthly during 2025, from late spring until the end of October.

Table 2. Number of monitored traps per site.

Plot ID	Town/Village (Province)	Cultivar	Plant density	Soil management	N. of internal traps	N. of extrenal traps	Total traps
1	Latiano (BR)	'Leccino'	LD	Tillage	5	20	25
2	Manduria (TA)	'Leccino'	LD	Tillage	7	10	17
3	Casarano (LE)	'Leccino'	LD	Mulching	4	14	18
4	Vernole (LE)	'Leccino'	LD	Mulching	4	12	16
5	Brindisi (BR)	'Leccino'	HD	Mulching	16	16	32
6	Brindisi (BR)	'Leccino'	HD	Tillage	8	20	28
7	Squinzano (BR)	'Leccino'	HD	Mulching	12	18	30
8	Galatina (LE)	'Leccino'	HD	Tillage	12	14	26
9	Trepuzzi (BR)	'Fs17'	SHD	Mulching	10	17	27
10	Squinzano (LE)	'Fs17'	SHD	Tillage	12	16	28
11	Nardò (LE)	'Fs17'	SHD	Tillage	10	15	25
12	Ugento (LE)	'Fs17'	SHD	Mulching	4	12	16
13	Otranto (LE)	'Fs17'	HD	Tillage	8	14	22
14	Alessano (LE)	'Fs17'	HD	Mulching	6	16	22
15	Ugento (LE)	'Fs17'	HD	Tillage	4	8	12
16	Acquarica (LE)	'Fs17'	HD	Mulching	4	12	16

BR = Brindisi; Le= Lecce; TA= Taranto

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2.2.2. *Xylella fastidiosa* symptoms severity scoring

Regular inspections were carried out in each olive grove to assess the presence of symptoms typically associated with *X. fastidiosa* infection and to evaluate the overall health status of the trees. Symptom severity was assessed by visual inspection using a six-class empirical rating scale: class 0 indicated symptomless trees; class 1 corresponded to initial desiccation affecting a few shoots (1–5 symptomatic twigs); class 2 indicated moderate desiccation involving several shoots (6–15 symptomatic twigs); class 3 referred to symptoms affecting an entire secondary branch; class 4 indicated desiccation of multiple secondary branches; and class 5 represented severe canopy decline, with whole branches showing extensive desiccation. During each assessment, all trees within each grove were visually inspected.

2.2.3 Monitoring *Xylella fastidiosa* infection status at field and tree levels

Data collected on juvenile and adult vector populations were used to classify olive groves into low, medium, or high vector-density classes, primarily based on the abundance of the main vector, *P. spumarius*. This classification was used to define the sampling intensity for *X. fastidiosa* monitoring: 50% of the trees were sampled in groves with low or medium vector density, whereas 30% were sampled in groves with high vector density (Table 1). The resulting classification integrated information from the monitoring programme but was primarily driven by the abundance of *P. spumarius*, the principal vector species.

Sampling was designed to estimate the proportion of infected trees and monitor infection dynamics over time at both the field and individual-tree levels. For field-level assessments, six shoots were collected from each sampled tree, representing different canopy orientations to ensure representative sampling.

To investigate within-tree infection dynamics, six infected trees were selected in each grove at the end of 2024. Eight canopy samples were collected from each tree at fixed positions representing both the lower and upper canopy and different canopy orientations. The same canopy positions were resampled at six-month intervals to monitor the temporal dynamics of *X. fastidiosa* infection within individual trees.

In all cases, *X. fastidiosa* detection was performed by real-time PCR following the protocol described by Harper et al. (2010). Total DNA was extracted from 0.5–0.8 g of olive shoot tissue prior to molecular analysis.

2.3 Statistical analysis

2.3.1 Analysis of vector abundance

Counts of juvenile and adult spittlebugs were analysed using generalized linear mixed-effects models (GLMMs) with a negative binomial error distribution and a log link function. A negative binomial distribution was selected because preliminary data exploration indicated overdispersion relative to a Poisson distribution. Site was included as a random effect to account for repeated observations collected from the same olive groves over different sampling dates and years.

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Juveniles and early-adults on ground-cover vegetation

For juvenile stages and early-season adults collected on ground-cover vegetation, observations were aggregated as the total number of individuals of each vector species recorded at each site and sampling date. The number of sampling units examined at each site and date was included in the models as an offset.

Three complementary analyses were performed.

First, differences in abundance between the two vector species were evaluated by testing the interaction among vector species, cultivar, and soil-management strategy.

Second, the effects of soil-management strategy, planting density, and their interaction were analysed separately for each cultivar and vector species. Separate analyses were required because the planting-density classes differed between the two cultivars ('Leccino': low and high density; 'Fs17': high and super-high density).

Third, the effects of sampling position (grove interior versus field border), soil-management strategy, and their interaction were analysed separately for each vector species.

Adult vectors

Adult populations were analysed separately for yellow sticky traps and sweep-net sampling because the two methods targeted different components of the adult population and differed in sampling effort.

To compare cultivars, soil-management strategies, and planting densities, counts were aggregated across all sampling dates within each site and year. This produced one annual abundance estimate for each site, year, and vector species. For yellow sticky traps, counts from internal and external traps were pooled. The number of trap observations or sweep-net samples contributing to each annual count was included as an offset. Year was treated as a repeated measure, while site was included as a random effect.

As for juvenile populations, an initial model evaluated the interaction among vector species, cultivar, and soil-management strategy. Separate models were then fitted for each cultivar and vector species to evaluate the effects of soil-management strategy, planting density, and their interaction.

To investigate seasonal variation in adult abundance, the monitoring period was divided into three biologically relevant periods according to the seasonal phenology of spittlebug populations under Apulian conditions:

- Early season: April to mid-June;
- Mid-season: mid-June to the end of August;
- Late season: September to October.

For yellow sticky traps, counts were aggregated by site, year, sampling period, trap position, and vector species. Models tested the interaction among sampling period, trap position, and soil-management strategy separately for each vector species. Trap position was classified as internal (within the grove) or external (along the grove border).

For sweep-net sampling, counts were aggregated by site, year, sampling period, and vector species. Models tested the interaction between sampling period and soil-management strategy separately for each vector species. In all analyses, the number of sampling units contributing to each aggregated count was included as an offset.

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Finally, the mean abundance of insect vectors estimated for each grove was used to classify the monitored groves into three vector-density classes (low, medium, and high). This classification was subsequently used as an explanatory variable in the analyses of X. fastidiosa infection incidence and infection dynamics.

2.3.2 Analysis of *Xylella fastidiosa* infection at field level

The incidence of *X. fastidiosa* infection was calculated for each grove and survey year as the proportion of infected trees relative to the total number of trees tested. Infection incidence was analysed using binomial generalized linear mixed-effects models (GLMMs). The response variable was specified as the number of infected and non-infected trees rather than as a percentage, thereby accounting for differences in sample size among groves. Site was included as a random intercept to account for repeated assessments of the same groves in 2024 and 2025.

Two sets of models were fitted. The first evaluated the interaction between cultivar and soil-management strategy. The second evaluated the interaction between cultivar and vector-density class. The effects of soil-management strategy, planting density, and their interaction were then analysed separately within each cultivar because planting-density classes differed between 'Leccino' and 'Fs17'.

The relative change in disease incidence between the two survey years (%ΔDI) was calculated according to Hornero et al. (2020) as:

$$\%\Delta DI = \frac{(DI_{2025} - DI_{2024})}{DI_{2024}} \times 100,$$

where $DI_{2024}$and DI_{2025} represent disease incidence in 2024 and 2025, respectively.

The %ΔDI values were natural log-transformed prior to analysis to improve the normality and homogeneity of variance of model residuals. The effects of cultivar, soil-management strategy, and their interaction were evaluated, together with the effects of cultivar, vector-density class, and their interaction. Because planting-density classes differed between cultivars, the effect of planting density was analysed separately within each cultivar.

2.3.3 Analysis of within-canopy infection

Within-canopy infection was expressed as the proportion of infected branches relative to the total number of branches tested for each tree and sampling occasion. These data were analysed using binomial generalized linear mixed-effects models. Individual trees were included as a random effect nested within site to account for both the grouping of trees within groves and repeated measurements of the same trees in autumn 2024, spring 2025, and autumn 2025. The models evaluated the interaction among sampling period, cultivar, and vector-density class.

All statistical analyses were performed using R version 4.5.2 (R Core Team, 2026). Negative binomial GLMMs were fitted using the glmmTMB package, whereas binomial GLMMs were fitted using lme4. Model adequacy was assessed using simulation-based residual diagnostics implemented in the DHARMa package. Diagnostic procedures included tests for residual uniformity, overdispersion, and zero inflation.

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For analyses based on linear models, the normality of residuals was assessed graphically and using the Shapiro–Wilk test, while homogeneity of variance was evaluated using the car package.

Whenever significant main effects or interactions were detected, estimated marginal means were calculated and pairwise comparisons among factor levels were performed using the emmeans package.

3 RESULTS

3.1 Vector abundance

3.1.1 Juveniles and early-adults on ground-cover vegetation

Across the three-year monitoring period, a total of 5,031 *P. spumarius* and 2,103 *N. campestris* juveniles and early-season adults were recorded on ground-cover vegetation across the 16 monitored olive groves. Population density varied markedly among groves and years (**Figures 2** and **3**). Mean *P. spumarius* density ranged from 0.13 to 40.33 insects m^{-2}, whereas *N. campestris* ranged from 0 to 17.16 insects m^{-2}.

Figures 2 and **3** highlight a high spatial variability among groves. Juvenile populations were generally higher in 'Leccino' groves than in 'Fs17' groves, particularly in low-density orchards managed with mulching (Fields 3 and 4), where both vector species reached the highest densities recorded during the study. In contrast, juvenile populations remained consistently low in most 'Fs17' groves, especially those managed under tillage.

Overall, *P. spumarius* was significantly more abundant than *N. campestris* across the monitored groves (**Figure 4**; $\chi^2 = 27.93$, df = 1, $P < 0.001$). The only exception was represented by tilled 'Leccino' groves, where the abundance of the two species did not differ significantly.

Soil-management strategy significantly affected juvenile vector abundance ($\chi^2 = 5.14$, df = 1, $P = 0.023$). Across cultivars, mulched groves supported higher juvenile densities (1.36 ± 0.33 insects m^{-2}) than tilled groves (0.37 ± 0.09 insects m^{-2}). Although this trend was consistent for both vector species, significant differences between mulching and tillage were detected only for *N. campestris* in 'Fs17' groves. No significant interactions among vector species, cultivar, and soil-management strategy were detected.

Planting density had a limited influence on juvenile abundance. In 'Leccino' groves, low-density orchards showed higher overall densities of both *P. spumarius* ($\chi^2 = 10.92$, df = 1, $P = 0.0010$) and *N. campestris* ($\chi^2 = 5.38$, df = 1, $P = 0.0204$) than high-density orchards. However, these differences were not confirmed when comparisons were performed separately within each soil-management strategy, indicating that the observed effect was largely associated with management rather than planting density. In 'Fs17' groves, juvenile densities were generally low regardless of planting density, and differences between high-density and super-high-density orchards were small.

Sampling position also influenced juvenile abundance (**Figure 5**). In tilled groves, both vector species were significantly less abundant within the grove than along unmanaged field borders ($\chi^2 = 6.97$, df = 1, $P = 0.0083$ for *P. spumarius*; $\chi^2 = 6.95$, df = 1, $P = 0.0084$ for *N. campestris*). This pattern is also evident in **Figures 2** and **3**, where border vegetation frequently supported larger juvenile populations than the managed ground cover within the grove.

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Under mulching, different responses were observed for the two vector species. *P. spumarius* showed similar densities inside the grove and along field borders, whereas *N. campestris* remained significantly more abundant in border vegetation. Overall, these results indicate that unmanaged field borders represent favourable habitats for juvenile spittlebugs, while mulching partially reduced the contrast between the grove interior and surrounding vegetation, particularly for *P. spumarius*.

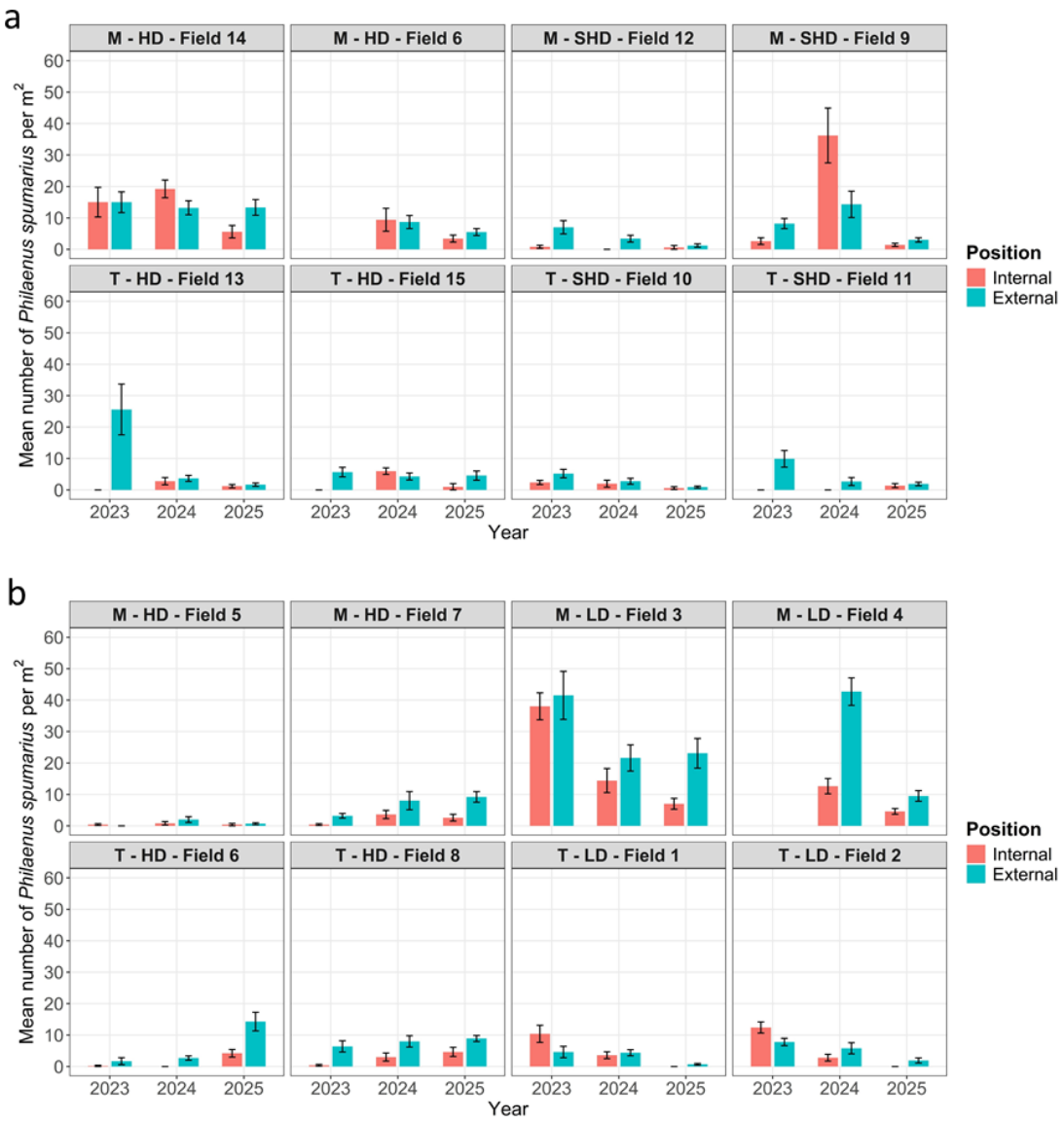


Figure 2. Mean (± SE) density of *Philaenus spumarius* juveniles and early-season adults on ground-cover vegetation in olive groves planted with (a) 'Fs17' and (b) 'Leccino' cultivars in Apulia, Italy. Red bars represent samples collected within the grove, whereas blue bars represent samples collected along field borders. M = mulching; T = tillage; LD = low-density groves; HD = high-density groves; SHD = super-high-density groves.

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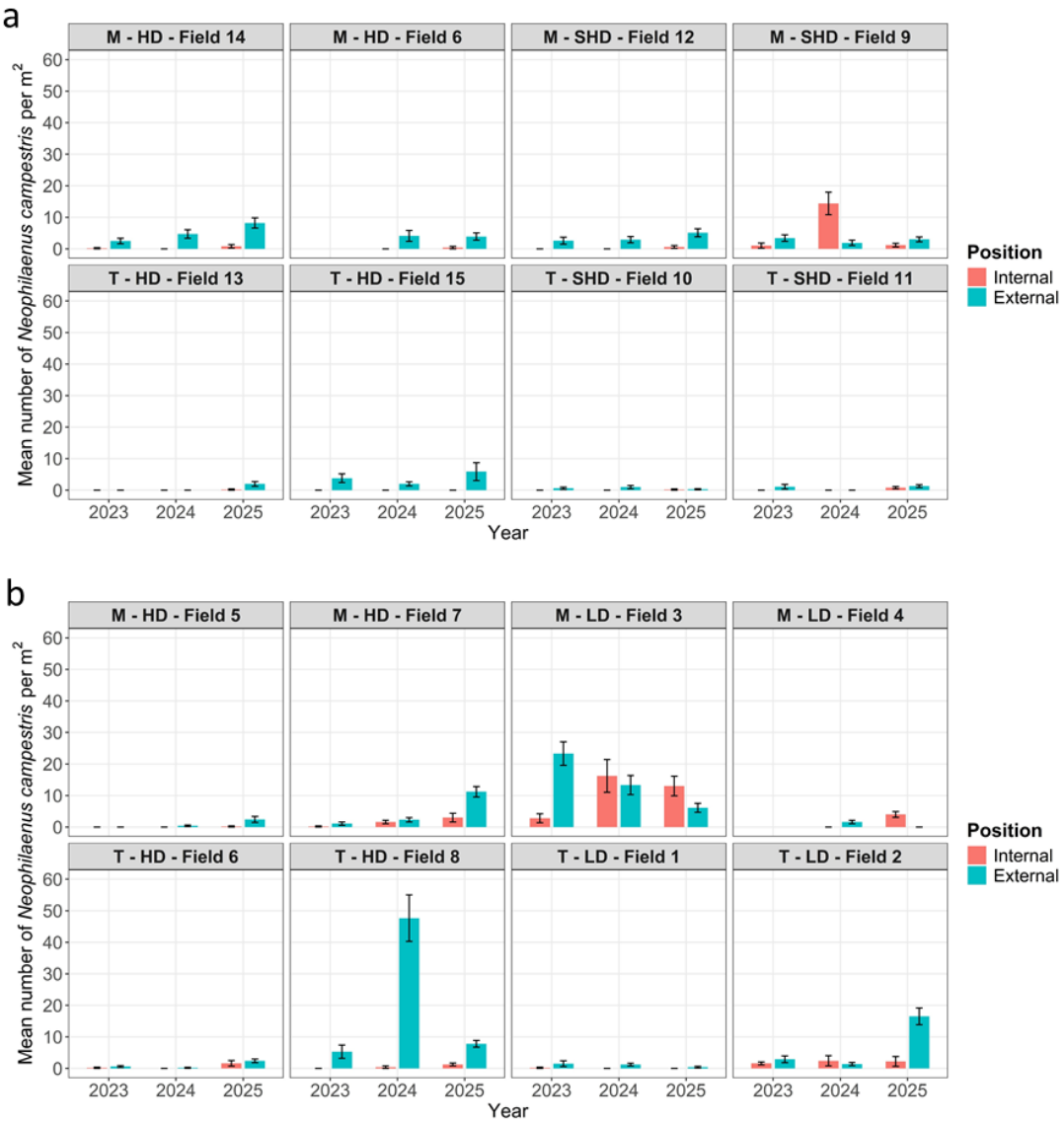


Figure 3. Mean (± SE) density of *Neophilaenus campestris* juveniles and early-season adults on ground-cover vegetation in olive groves planted with (a) 'Fs17' and (b) 'Leccino' cultivars in Apulia, Italy. Red bars represent samples collected within the grove, whereas blue bars represent samples collected along field borders. M = mulching; T = tillage; LD = low-density groves; HD = high-density groves; SHD = super-high-density groves.

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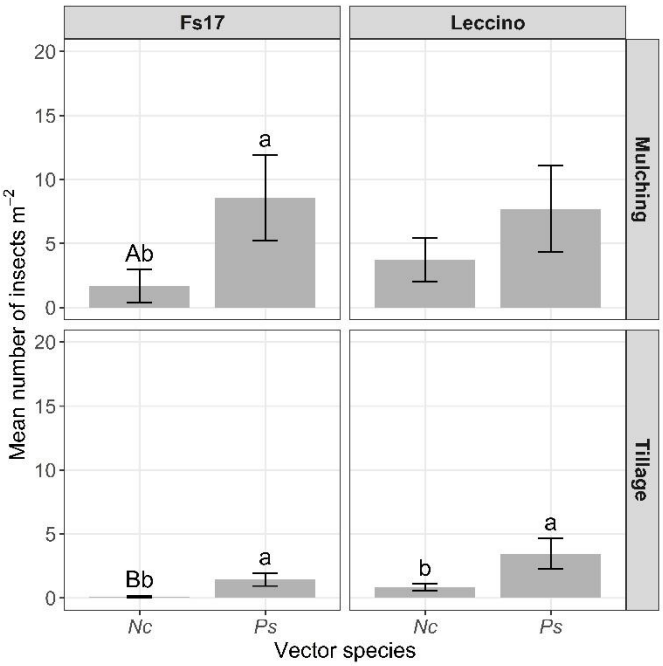


Figure 4. Mean (± SE) density of *Philaenus spumarius* (Ps) and *Neophilaenus campestris* (Nc) juveniles and early-season adults on ground-cover vegetation in olive groves planted with the 'Fs17' and 'Leccino' cultivars in Apulia, Italy. Different lowercase letters indicate significant differences among vector species within each cultivar × soil-management combination (Tukey-adjusted pairwise comparisons, α = 0.05).

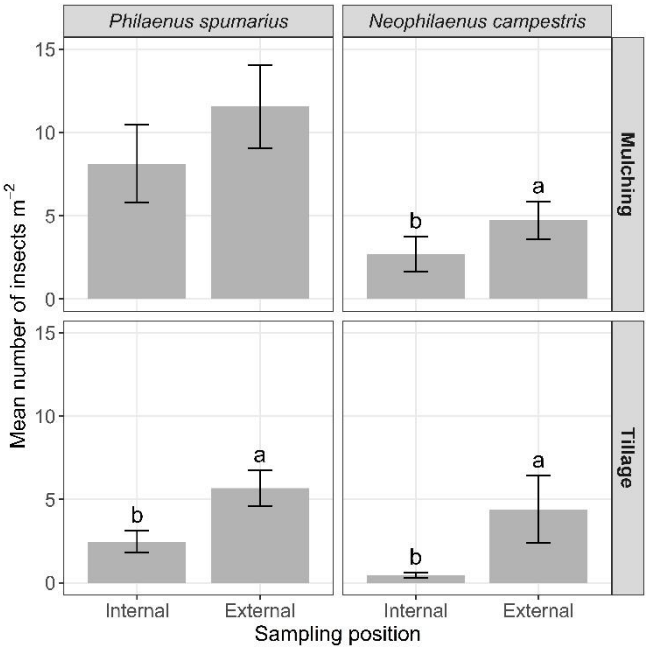


Figure 5. Mean (± SE) density of (a) *Philaenus spumarius* and (b) *Neophilaenus campestris* juveniles and early-season adults on ground-cover vegetation in internal and border areas of olive groves managed with mulching or tillage in Apulia, Italy. Different lowercase letters indicate significant differences between sampling positions within each soil-management treatment, whereas different uppercase letters indicate significant differences between soil-management strategies within each sampling position (Tukey-adjusted pairwise comparisons, α = 0.05).

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3.1.2 Adult vectors

Adult vector populations were monitored using yellow sticky traps installed in the olive canopy and sweep-net sampling on ground-cover vegetation. The two monitoring methods provided complementary information on adult abundance and seasonal dynamics.

Overall, no significant differences were detected between *P. spumarius* and *N. campestris* adult abundance in yellow sticky-trap captures (**Figure 6a**; χ^2 = 1.44, df = 1, *P* = 0.230). Likewise, adult abundance was not significantly affected by cultivar, soil-management strategy, or their interactions (all *P* > 0.05).

In contrast, sweep-net sampling revealed a significant effect of soil management on adult abundance (χ^2 = 9.57, df = 1, *P* = 0.002). Both vector species were consistently more abundant on ground-cover vegetation in mulched than in tilled groves (**Figure 6b**), with overall mean abundances of 0.086 ± 0.021 and 0.022 ± 0.003 adults sweep^{-1}, respectively. Significant differences between soil-management strategies were detected only in 'Fs17' groves for both vector species, although the same trend was observed in 'Leccino' groves. Neither vector species nor cultivar significantly affected adult abundance in sweep-net samples, and no significant interactions among the tested factors were detected.

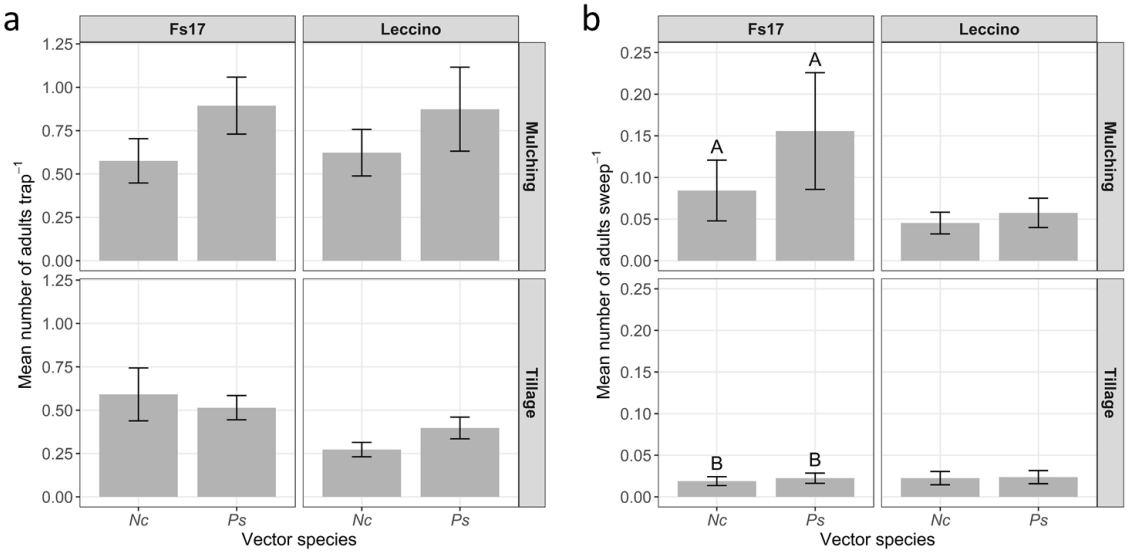


Figure 6. Mean (± SE) abundance of adult *Philaenus spumarius* (Ps) and *Neophilaenus campestris* (Nc) recorded in olive groves planted with 'Fs17' and 'Leccino' under mulching or tillage. Adults were monitored using (a) yellow sticky traps installed in the canopy and (b) sweep-net sampling on ground-cover vegetation. Different uppercase letters indicate significant differences between soil-management strategies within each vector species (Tukey-adjusted pairwise comparisons, α = 0.05).

The effect of planting density differed between cultivars and vector species. In 'Fs17' groves, planting density did not influence *P. spumarius* abundance, irrespective of the sampling method (sticky traps: χ^2 = 0.33, df = 1, *P* = 0.568; sweep nets: χ^2 = 1.47, df = 1, *P* = 0.225). Conversely, *N. campestris* was consistently more abundant in super-high-density (SHD) than in high-density (HD) groves, both in sticky-trap captures (χ^2 = 28.12, df = 1, *P* < 0.001) and sweep-net samples (χ^2 = 5.19, df = 1, *P* = 0.023).

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In 'Leccino' groves, planting density significantly affected adult abundance. Low-density (LD) groves consistently supported higher numbers of *P. spumarius* than high-density (HD) groves, both in canopy traps ($\chi^2 = 30.40$, df = 1, $P < 0.001$) and on ground-cover vegetation ($\chi^2 = 4.39$, df = 1, $P = 0.032$). A similar pattern was observed for *N. campestris*, although the effect was statistically significant only for sticky-trap captures ($\chi^2 = 22.75$, df = 1, $P < 0.001$), while differences detected by sweep-net sampling were marginal ($\chi^2 = 3.30$, df = 1, $P = 0.069$).

Seasonal dynamics were evident for both vector species (**Figures 7** and **8**). Yellow sticky-trap captures showed a clear seasonal decline in adult abundance throughout the monitoring period (**Figure 7**). Adult abundance was highest during the early season and progressively declined during the mid- and late-season sampling periods. This pattern was particularly evident for adults collected on ground-cover vegetation, where both *P. spumarius* and *N. campestris* showed significantly higher abundance during the early season than later in the year.

For canopy trap captures, *P. spumarius* abundance was significantly affected by sampling period ($\chi^2 = 35.29$, df = 2, $P < 0.001$), with a significant interaction between sampling period and soil-management strategy ($\chi^2 = 29.90$, df = 2, $P < 0.001$). In mulched groves, captures were significantly higher during the early season than during the mid- and late-season periods, whereas seasonal differences were less pronounced under tillage (**Figure 7a**). *N. campestris* abundance also varied significantly among sampling periods ($\chi^2 = 85.31$, df = 2, $P < 0.001$; **Figure 7b**). Although the interaction between sampling period and soil-management strategy was only marginally significant ($\chi^2 = 5.96$, df = 2, $P = 0.051$), no overall effects of soil-management strategy or trap position were detected.

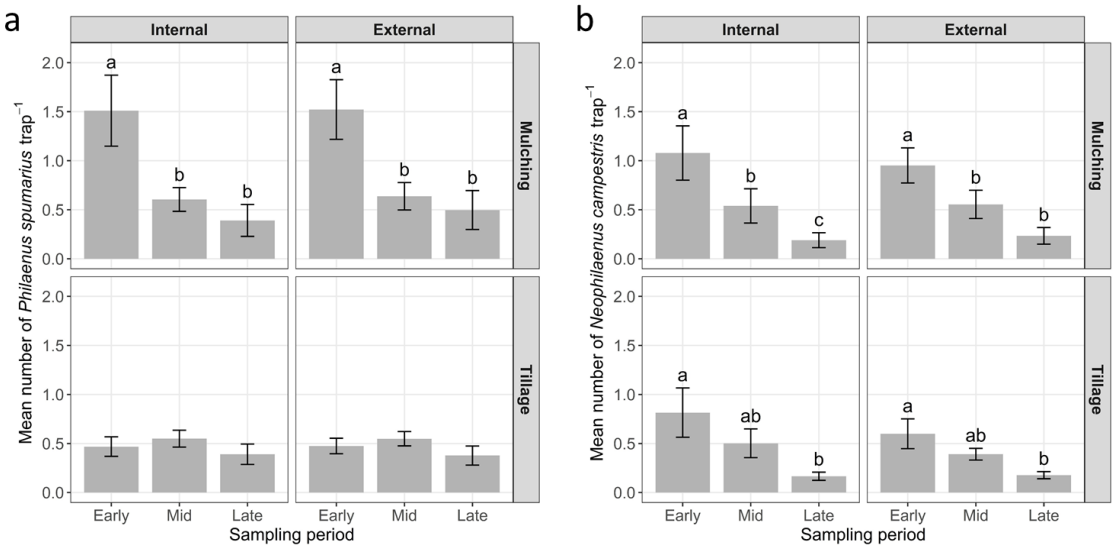


Figure 7. Mean (± SE) abundance of adult (a) *Philaenus spumarius* and (b) *Neophilaenus campestris* captured per yellow sticky trap during the three sampling periods in olive groves managed with mulching or tillage. Different letters indicate significant differences among sampling periods (Tukey-adjusted pairwise comparisons, $\alpha = 0.05$).

Adult abundance on ground-cover vegetation, estimated by sweep-net sampling, was significantly affected by both sampling period and soil-management strategy for *P. spumarius* (sampling period: $\chi^2 = 38.07$, df = 2, $P < 0.001$; soil management: $\chi^2 = 6.83$, df = 1, $P = 0.0090$)

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and *N. campestris* (sampling period: $\chi^2 = 57.83$, df = 2, $P < 0.001$; soil management: $\chi^2 = 5.49$, df = 1, $P = 0.0192$).

As shown in **Figure 8**, both vector species exhibited a similar seasonal pattern on ground-cover vegetation. Adult abundance was highest during the early season and declined markedly during the mid- and late-season sampling periods under both soil-management strategies. In both species, adult abundance was significantly higher in mulched than in tilled groves during the early sampling period. For *P. spumarius*, this difference remained significant during the mid-season, whereas for *N. campestris* no significant differences between soil-management strategies were detected after the early season. Overall, these results indicate that mulched ground-cover vegetation supports higher adult vector abundance mainly during the spring period, whereas differences between soil-management strategies progressively disappear later in the season as adult populations decline.

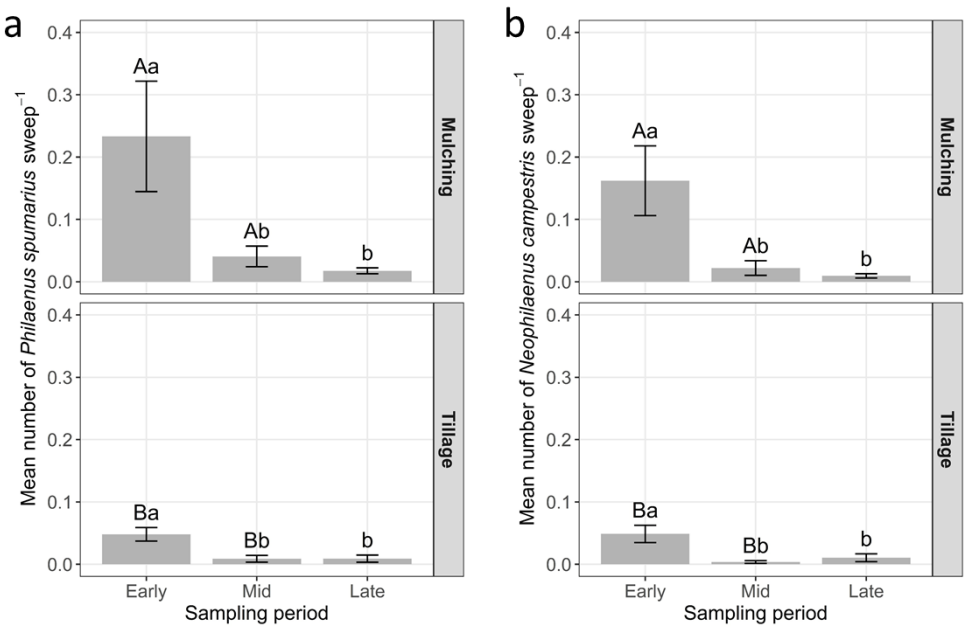


Figure 8. Mean (± SE) abundance of adult (a) *Philaenus spumarius* and (b) *Neophilaenus campestris* collected per sweep-net sample during the three sampling periods in olive groves managed with mulching or tillage. Different lowercase letters indicate significant differences among sampling periods within each soil-management strategy, whereas different uppercase letters indicate significant differences between soil-management strategies within each sampling period (Tukey-adjusted pairwise comparisons, $\alpha = 0.05$).

3.2 Analysis of *Xylella fastidiosa* infection at field level

A marked variability in *X. fastidiosa* infection incidence was observed among the monitored olive groves (**Figure 9**). Field-level incidence ranged from 1.68% in a 'Leccino' grove to 69.45% in an 'Fs17' grove. Despite this variability, disease incidence increased from 2024 to 2025 in all monitored groves.

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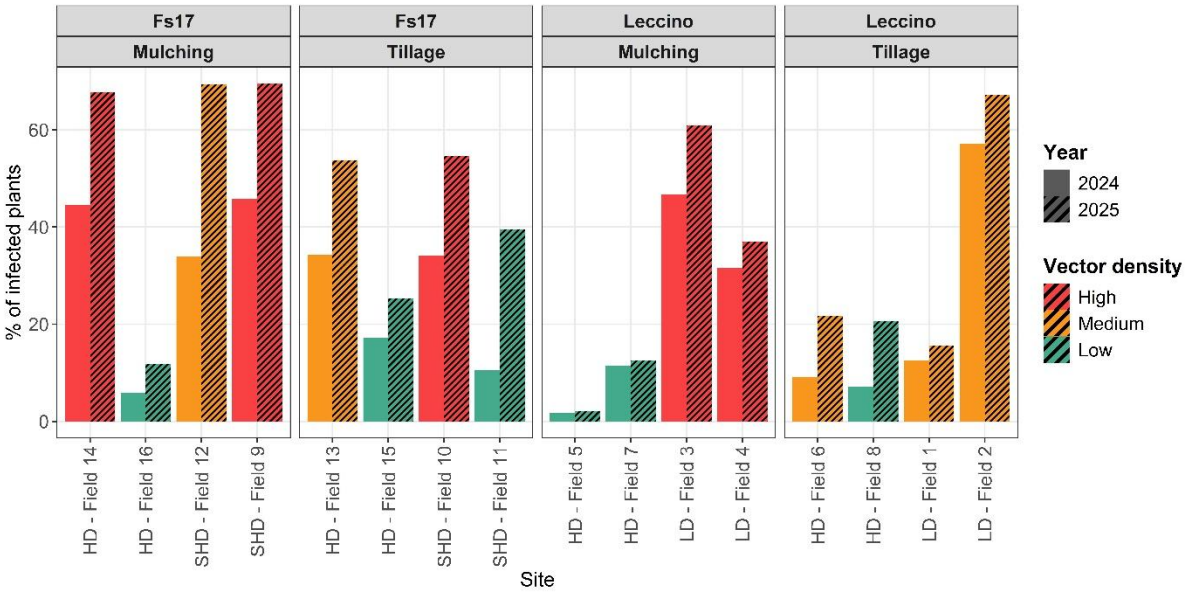


Figure 9. Incidence (%) of *Xylella fastidiosa* infection in olive trees of the resistant cultivars 'Fs17' and 'Leccino' during the 2024 and 2025 surveys in 16 olive groves in Apulia, Italy, managed with mulching or tillage. Colours indicate the vector-density class assigned to each grove based on monitoring data.

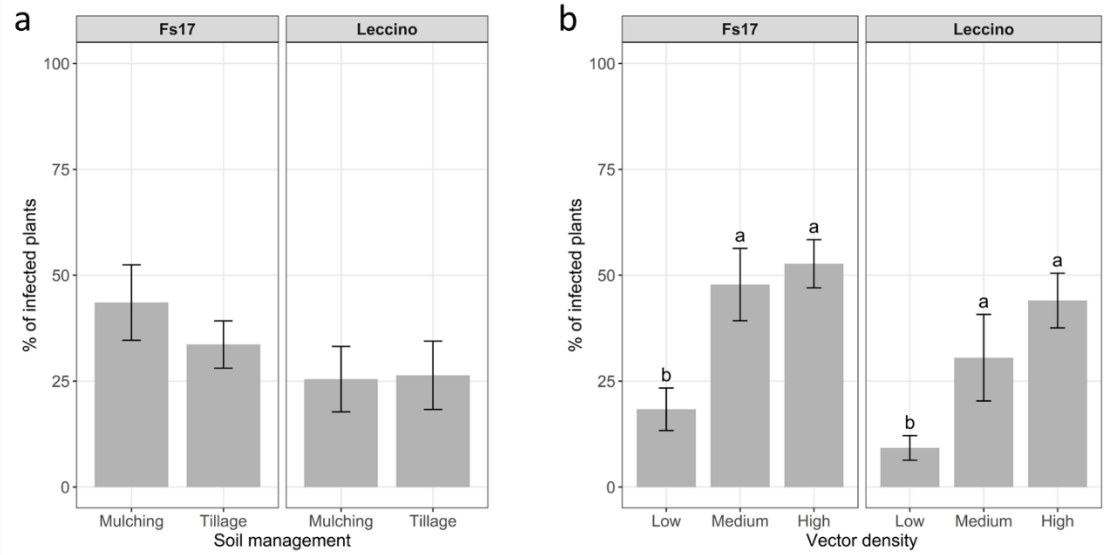


Figure 10. Mean (± SE) incidence of *Xylella fastidiosa* infection in olive trees of the resistant cultivars 'Fs17' and 'Leccino' during the 2024 and 2025 surveys in 16 olive groves in Apulia, Italy. (a) Effects of cultivar and soil-management strategy. (b) Effects of cultivar and vector-density class. Different lowercase letters indicate significant differences among soil-management treatments or vector-density classes, respectively (Tukey-adjusted pairwise comparisons, α = 0.05).

Neither cultivar nor soil-management strategy significantly affected disease incidence when evaluated together (cultivar: $\chi^2 = 2.39$, df = 1, $P = 0.122$; soil management: $\chi^2 = 0.002$, df = 1, $P = 0.962$; **Figure 10a**). However, when disease incidence was analysed in relation to vector-density class, both cultivar ($\chi^2 = 5.18$, df = 1, $P = 0.023$) and vector density ($\chi^2 = 25.61$, df = 2,

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P < 0.001) had significant effects (**Figure 10b**). Overall, estimated disease incidence was higher in 'Fs17' than in 'Leccino' groves (38.6 ± 5.2% and 25.9 ± 5.4%, respectively), while groves classified as having medium or high vector density showed markedly higher incidence than groves with low vector density. However, post-hoc comparisons did not detect significant differences between cultivars within any vector-density class.

The effect of planting density differed between cultivars. In 'Fs17' groves, planting density did not significantly influence disease incidence (χ^2 = 1.54, df = 1, *P* = 0.215). In contrast, disease incidence was significantly higher in low-density than in high-density 'Leccino' groves (χ^2 = 11.86, df = 1, *P* < 0.001).

The relative increase in disease incidence between 2024 and 2025 (%ΔDI) also varied substantially among groves (**Figure 11**). The highest variability was observed among groves classified as having low vector density, where %ΔDI ranged from 9.7% to 275%. By comparison, groves with high vector density showed a narrower range of relative increase (17.4–52.2%).

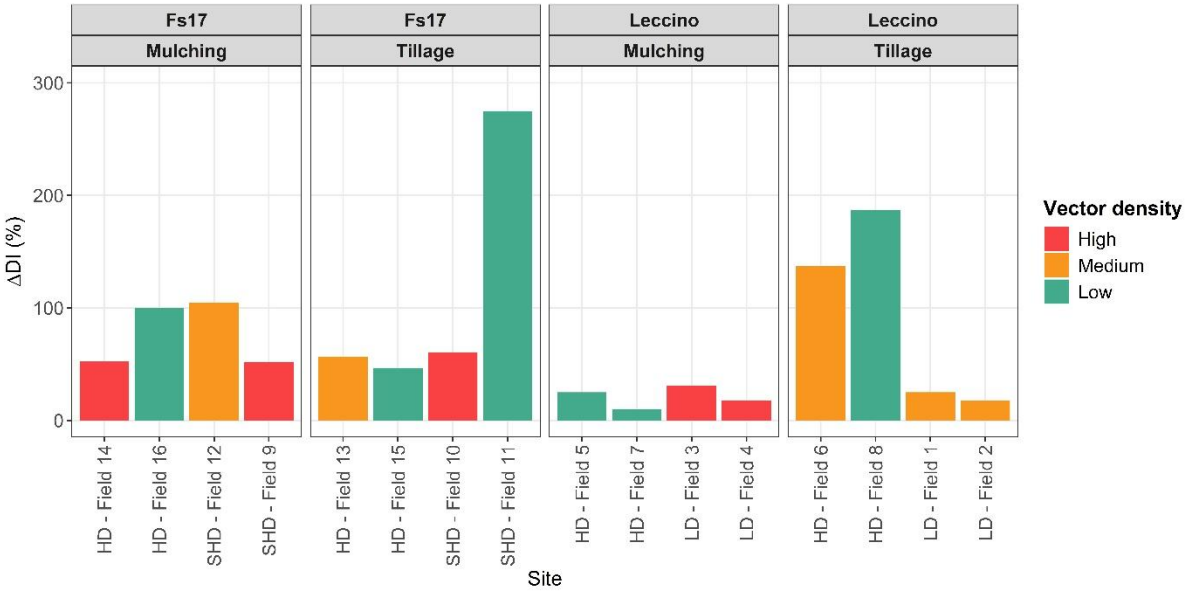


Figure 11. Relative change in *Xylella fastidiosa* disease incidence (%ΔDI) between 2024 and 2025 in the 16 monitored olive groves in Apulia, Italy. Colours indicate the vector-density class assigned to each grove.

No significant effects of soil-management strategy, vector-density class or planting density were detected on %ΔDI (all *P* > 0.05; **Figure 12**). A weak effect of cultivar was observed (F = 4.56, df = 1, *P* = 0.054), with a greater mean increase in disease incidence in 'Fs17' groves (93.4 ± 27.1%) than in 'Leccino' groves (56.2 ± 23.7%).

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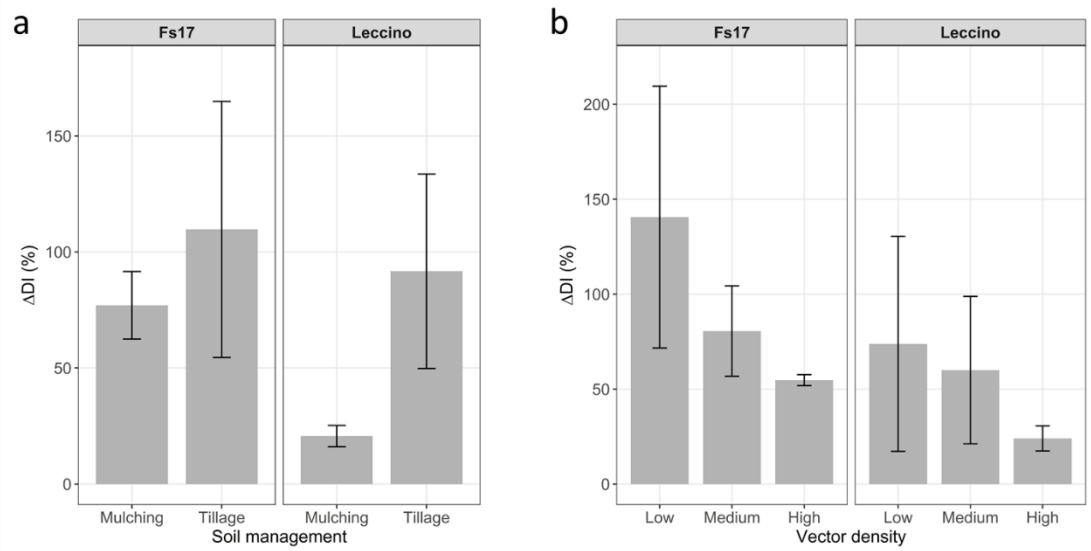


Figure 12. Mean (± SE) relative change in *Xylella fastidiosa* disease incidence (%ΔDI) between 2024 and 2025 in olive groves planted with the resistant cultivars 'Fs17' and 'Leccino' in Apulia, Italy. (a) Effects of cultivar and soil-management strategy. (b) Effects of cultivar and vector-density class.

Despite qPCR-confirmed infections, visual symptoms were generally absent or very limited. Mild symptoms (severity classes 1 and 2) were recorded only in Field 4 ('Leccino'), where 27.6% of trees showed symptoms, and in Field 13 ('Fs17'), where symptoms were observed in 16.9% of trees during the 2024 survey. No symptomatic trees were recorded in the remaining groves during the study period.

3.3 Analysis of within-canopy infection

A total of 91 infected olive trees were selected to investigate the within-canopy distribution of *Xylella fastidiosa*, including 46 'Fs17' and 45 'Leccino' trees. Each tree was monitored over three sampling periods by analysing eight fixed canopy sections.

The proportion of infected canopy samples varied markedly among trees and over time (**Figure 13**). During the first two sampling periods, more than half of the trees of both cultivars showed infection in less than 25% of the sampled canopy sections. Trees with extensive within-canopy infection (>75% of infected canopy samples) represented less than 7% of the monitored plants at the first assessment. By the final survey, this proportion had increased to approximately 32% in 'Fs17', whereas it remained below 10% in 'Leccino'.

Successive analyses of the same canopy sections revealed a highly heterogeneous and dynamic distribution of *X. fastidiosa* within infected trees. Between autumn 2024 and spring 2025, only 29.6% of previously infected canopy samples in 'Fs17' and 29.2% in 'Leccino' remained positive. Between spring and autumn 2025, persistence increased to 66.3% and 51.3%, respectively. Considering the entire monitoring period, 60.8% of canopy samples that tested positive in autumn 2024 were again positive in autumn 2025 in 'Fs17', compared with 40.0% in 'Leccino'.

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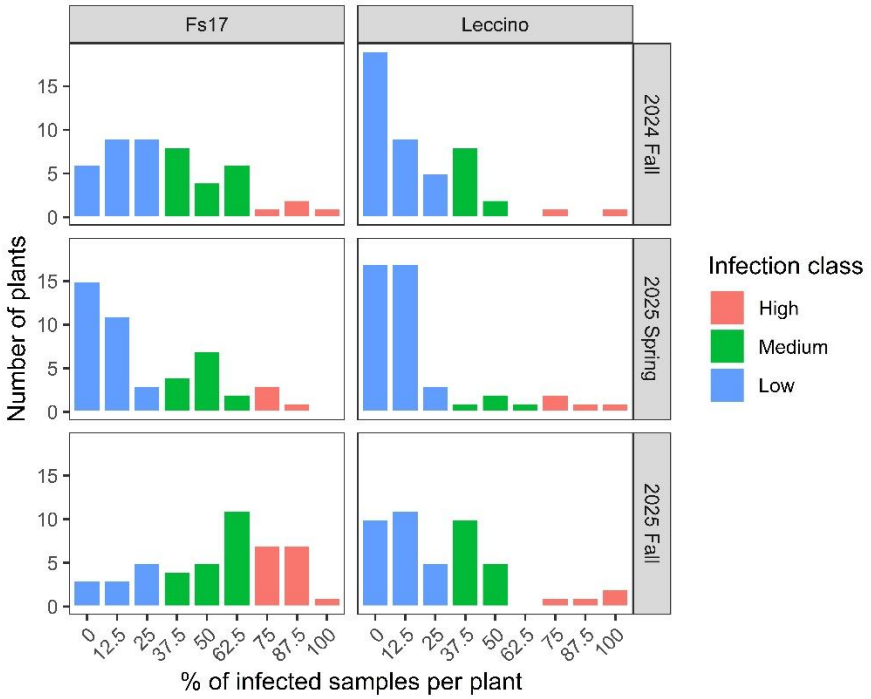


Figure 13. Distribution of infected canopy samples in 'Fs17' and 'Leccino' olive trees over three sampling periods. Histograms show the number of trees according to the percentage of infected canopy samples.

Within-canopy infection was significantly affected by cultivar (χ^2 = 7.09, df = 1, P = 0.0077), sampling period (χ^2 = 54.23, df = 2, P < 0.001), and the interaction among cultivar, sampling period, and vector-density class (χ^2 = 14.55, df = 4, P = 0.0057) (**Figure 14**).

In 'Fs17', the proportion of infected canopy samples decreased significantly from autumn 2024 to spring 2025 in groves with medium and high vector density, followed by a significant increase from spring to autumn 2025 across all vector-density classes. In contrast, 'Leccino' showed a more stable infection pattern. Although a gradual increase in within-canopy infection was observed in groves with low and medium vector density, differences among sampling periods were not significant. A significant increase was detected only in the high vector-density class between spring and autumn 2025.

Comparisons between cultivars showed no significant differences during the first two sampling periods. However, by autumn 2025, 'Fs17' exhibited significantly higher within-canopy infection than 'Leccino'. No significant differences among vector-density classes were detected within any sampling period.

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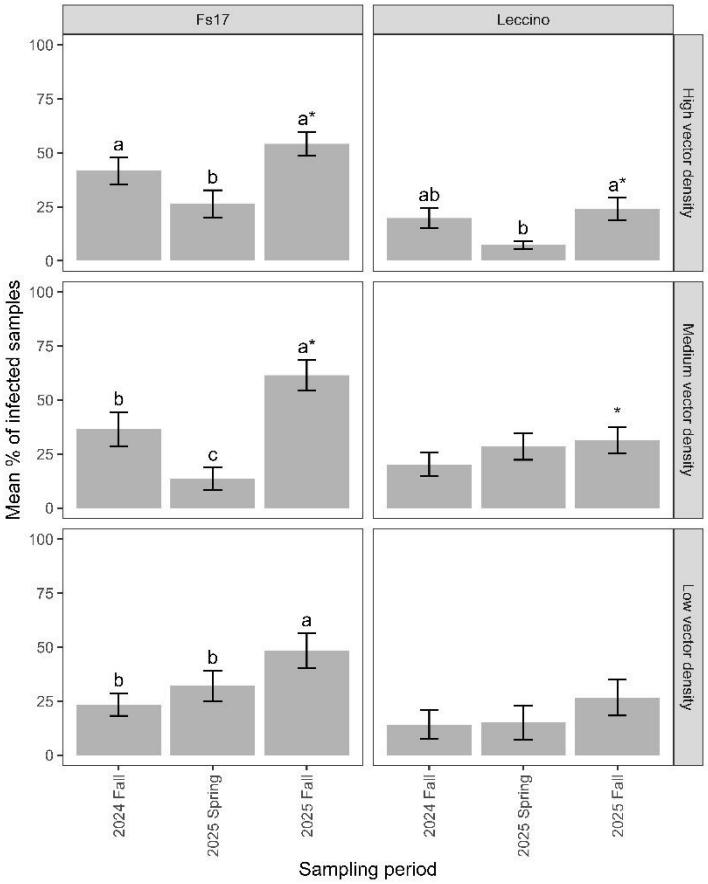


Figure 14. Mean (± SE) proportion of *Xylella fastidiosa*-infected canopy samples in 'Fs17' and 'Leccino' olive trees over three sampling periods in groves classified according to vector-density class. Different lowercase letters indicate significant differences among sampling periods within each cultivar × vector-density combination. Asterisks indicate significant differences between cultivars within the same sampling period and vector-density class (Tukey-adjusted pairwise comparisons, α = 0.05).

4 SUMMARY OF RESULTS AND CONCLUSIONS

Monitoring of spittlebug vectors over three consecutive years revealed marked spatial and temporal variability among the monitored olive groves. Juvenile and early-season adult populations on ground-cover vegetation were dominated by *P. spumarius*, which generally occurred at higher densities than *N. campestris*. Soil-management strategy significantly influenced vector abundance, with consistently higher juvenile and adult densities on ground-cover vegetation in mulched than in tilled groves. Differences between managed groves and unmanaged field borders further demonstrated the importance of border vegetation as a reservoir for vector populations, indicating that effective vector suppression should include both within-grove management and the surrounding vegetation.

Adult populations followed a clear seasonal pattern, with maximum abundance during the early season and a progressive decline thereafter. Sweep-net sampling showed that mulched ground-cover vegetation supported higher adult abundance during spring, whereas yellow sticky traps installed in the olive canopy detected no overall effect of soil-management strategy. These results indicate that the effect of soil management is mainly expressed while

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adult vectors are associated with ground-cover vegetation, whereas no overall effect was detected once adults were monitored in the olive canopy.

Xylella fastidiosa infection incidence varied considerably among groves, ranging from 1.68% to 69.45%, and increased between 2024 and 2025 in every monitored grove. Although soil-management strategy did not significantly affect disease incidence, both cultivar and vector-density class were significantly associated with infection levels. Groves classified as having medium or high vector density consistently showed higher infection incidence than those with low vector density, confirming vector pressure as one of the main drivers of epidemic development even in plantations established with resistant cultivars. Overall, 'Fs17' groves showed higher infection incidence than 'Leccino' groves. Planting density influenced disease incidence only in 'Leccino', where low-density groves showed higher infection levels than high-density groves, consistent with the higher abundance of adult vectors recorded in these orchards. By contrast, the annual increase in disease incidence (%ΔDI) showed large variability among groves and was not significantly explained by soil-management strategy, vector-density class or planting density.

Despite the widespread detection of *X. fastidiosa* by qPCR, symptom expression remained limited throughout the study. Mild symptoms were observed in only two of the sixteen monitored groves, confirming that resistant cultivars effectively limit symptom development, although they do not prevent bacterial infection or subsequent disease spread.

Within-canopy analyses demonstrated that bacterial distribution within infected trees is highly heterogeneous and temporally dynamic. Most infected trees showed positive results in fewer than 25% of the sampled canopy sections during the first monitoring period, whereas the proportion of highly infected trees increased over time, particularly in 'Fs17'. Repeated sampling of the same canopy sections showed that bacterial detection frequently changed over time, indicating that *X. fastidiosa* is unevenly distributed within resistant trees and that repeated sampling is essential for reliable diagnosis and epidemiological studies.

Field monitoring of the selected olive groves is continuing beyond the project duration and will provide an increasingly robust dataset for evaluating the long-term epidemiology of *X. fastidiosa* in resistant olive plantations.

Overall, the results demonstrate that resistant cultivars represent the cornerstone of olive-grove regeneration in infected areas of Apulia, but they are not sufficient as a stand-alone strategy to prevent infection spread. Long-term durability of resistant plantations depends on the combined effects of cultivar response, vector pressure, management of ground-cover vegetation and field borders, planting system, and the surrounding epidemiological context. Sustainable regeneration of olive production therefore requires integrated, site-specific management strategies combining resistant cultivars with continuous vector suppression, reduction of local inoculum sources, appropriate management of ground vegetation and field margins, and long-term epidemiological monitoring.

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