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Ground Cover Management in Olive Groves Reduces Populations of *Philaenus spumarius* (Hemiptera: Aphrophoridae), Vector of *Xylella fastidiosa*

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Abstract

Philaenus spumarius (Linnaeus, 1758) (Hemiptera: Aphrophoridae) is considered the main vector of *Xylella fastidiosa* (Wells Raju et al. 1986) (Xathomonadales: Xanthomonadaceae), agent of the Olive Quick Decline Syndrome in Southern Europe. To limit the spread of the disease, it is of primary importance to identify effective control measures against the vector. Besides chemical control, cultural practices could potentially help reducing vector activity and population density. Here, we tested the effectiveness of three different ground cover management practices in controlling vector populations in olive groves in the Abruzzo region (Central Italy). We compared tillage (two tillage operations in spring followed by two cuts in summer), frequent mowing (four cuts from spring to summer) and a control (two cuts in summer) by sampling vectors both in the ground vegetation and in the tree canopy. In late spring, after the peak of the population, tillage reduced *P. spumarius* density by 60%, while frequent mowing only reduced the density by 20% compared to control plots. The differences tended to disappear with time. The treatments had the same effect on the vector density in both the ground vegetation and tree canopy. The vectors were more concentrated in the ground cover at the beginning of the season while in summer both the canopy and ground vegetation had the same vector density. Our findings suggest that tillage is a viable option for the containment of *P. spumarius*, as frequent mowing did not achieve sufficient control efficacy.

Key words: meadow spittlebug, olive quick decline syndrome, grass mowing, tillage

Xylella fastidiosa (Wells Raju et al. 1986) (Xathomonadales: Xanthomonadaceae) is a Gram-negative xylem-limited bacterium native in the American continent and transmitted by a wide range of xylem fluid-feeding sharpshooters and spittlebugs (Almeida et al. 2005, Cornara et al. 2019). *Xylella fastidiosa* spread is strictly related to the presence, behavior, and abundance of its vectors (Irwin and Ruesink 1986, Stafford et al. 2011, Mauck et al. 2018). In Europe, *X. fastidiosa* has been officially present since 2013, when the subsp. *pauca* was reported in the Apulia region (Italy) causing the Olive Quick Decline Syndrome (OQDS) (Saponari et al. 2013). *Xylella fastidiosa* is mainly transmitted by the meadow spittlebug *Philaenus spumarius* (L.) (Saponari et al. 2014, Cornara et al. 2017, White et al. 2017). Moreover, adults of *Neophilaenus campestris* (Fallén, 1805) (Hemiptera: Aphrophoridae) and *Philaenus italosignus* (Drosopoulos and Remane, 2000) (Hemiptera: Aphrophoridae) are also able to transmit the disease even if their population density and transmission efficiency is lower than those of *P. spumarius* (Cavalieri et al. 2019). *Philaenus spumarius* is a monovoltine, widely polyphagous species widespread in the Boreal hemisphere. In south Europe, its juvenile stages are present from March to May (Morente et al. 2018) on forbs and graminoids (Yurtsever 2000, Biedermann and Niedringhaus 2009, Latini et al. 2019, Bodino et al. 2020). The adults of the vector, generally, reside beneath the canopy of trees and bushes from June and throughout the dry periods. In September, they return to the ground for laying eggs (Lopes et al. 2014, Cornara et al. 2017, Cruaud et al. 2018, Morente et al. 2018, Antonatos et al. 2019; Bodino et al. 2019). As movement of *P. spumarius* juveniles is limited to a few meters throughout their development and only on herbaceous plants they cannot transmit the disease to olive trees (Cornara 2018, Bodino 2020). At a larger spatial scale, landscapes with greater cover of olive groves support larger populations of *P. spumarius* indicating that this habitat is particularly suitable for the vector (Santoiemma et al. 2019). Hence, developing effective control measures against the vectors within olive groves appears of utmost importance to slow down *X. fastidiosa* spread.

In the OQDS outbreak areas, the use of broad-spectrum insecticides against *P. spumarius* adults in early summer, before canopy colonization, is mandatory. However, few available active ingredients such as deltamethrin and acetamiprid, are effective against the vector and are characterized by low persistence (Dongiovanni et al. 2018). Considering that agro-chemicals have several negative side effects on agro-ecosystems (Rodríguez et al. 2003), it is crucial to find more sustainable and ecofriendly control strategies to reduce adult vector density and to prevent its canopy colonization. Furthermore, several plant species occurring within the olive groves are both inoculum sources of *X. fastidiosa* and *P. spumarius* host plants (e.g., *Vinca* spp. (Linnaeus, 1753) (Gentianales: Apocynaceae), *Erigeron* spp. (Linnaeus, 1753) (Asterales: Asteraceae), *Asparagus acutifolius* (Linnaeus, 1753) (Asparagales: Asparagaceae)) (EFSA 2020) potentially contributing to increase the risks of infection. Hence, the management of the ground vegetation is expected to affect vector density and behavior.

Disturbance of grassland vegetation is well known to strongly reduce populations of arthropods (Humbert et al. 2009, 2010; Unterweger et al. 2017) and therefore could also affect negatively the populations of juvenile spittlebugs through direct mortality. Considering that, in olive groves, no detailed experimental data on the influence of grass cover management on the population density of *P. spumarius* is available, the aims of this work were to 1) investigate the impact of three ground cover management regimes (2 spring tillage + 2 summer cuts vs 2 spring cuts + 2 summer cuts vs 2 summer cuts) on the reduction of *P. spumarius* population density, and 2) monitor the season movement of *P. spumarius* from the ground vegetation to the tree canopies under the different treatments. Our results will help develop cultural techniques to reduce vector abundance both in OQDS infested area and in areas where *X. fastidiosa* is not present, in order to limit the spread of the disease with low environmental impact techniques.

Materials and Methods

Study Area and Sampling Design

The study area was located in southern Italy (Abruzzo Region), where the climate is transitional between continental and Mediterranean (Santoiemma et al. 2019). We selected 13 olive groves from 40 to 300 m above sea level, characterized by a plant density ranging from 110 to 330 trees/ha (mean 207 trees/ha, SD 73.4). Cultivars were Dritta (50%), Leccino (30%), Nebbio, and Gentile di Chieti (10% each). Ground cover is generally managed by mowing spontaneous vegetation twice a year in late spring and summer. The mowing is generally suspended during summer because vegetation dries out due to drought conditions. Olive groves were treated with two insecticide applications using dimethoate in late summer/early autumn against *Bactrocera oleae* (Rossi, 1790) (olive fly; Diptera: Tephrytidae) upon reaching 8–10% of infested drupes. The substance is considered poorly effective against *P. spumarius* (Dongiovanni et al. 2018). All olive groves were nonirrigated.

In each orchard, based on the dispersal capability of *P. spumarius* (Weaver and King 1954, Strona et al. 2017), we selected three large plots (50 × 50 m) to apply a different ground cover treatment each. The size of the plots was intended to minimize movement of individuals between treatments. The three grass cover managements were the following: 1) tillage, 2) frequent mowing, and 3) control (Fig. 1).

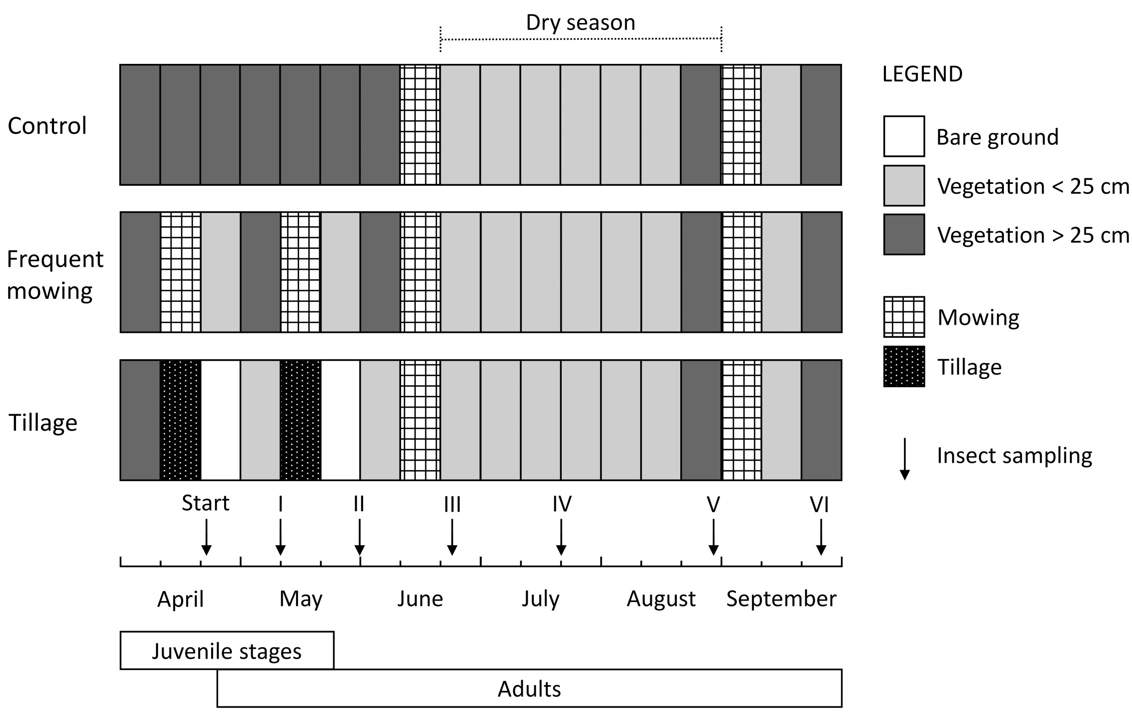

Fig. 1. Ground cover management in the three plots (control, frequent mowing, and tillage) over time in each olive grove. Grass development and phenology of *Philaenus spumarius* are also reported.

Tillage included two superficial soil tillage operations in early spring followed by two cuts in late spring and late summer. Both tillage operations were performed when *P. spumarius* was at the juvenile stages. Frequent mowing included two cuts in early spring followed by two cuts in late spring and summer. Control plots included two cuts, in late spring and summer, and represent the standard practice in the study area.

Insect Sampling

In each experimental plot, the presence and abundance of adults of the known vectors of *X. fastidiosa* were sampled using eight handmade transparent traps (21 × 29.7 cm), for a total of 24 traps per olive orchard. The traps were made by spraying non-drying sticky glue (VeBio Vebicolla) on transparent A4 300 g/m^2 PVC sheets. The traps were placed in the inner part of the plots, on four trees around the centre of each plot. On each tree, to check the movement of the vectors between ground cover and canopies, one trap was positioned at 0.5 m from the ground level ('ground') and one in the canopy at 2–2.5 m ('canopy'). The sampling started on April 22nd and ended on September 26th, 2019, replacing the traps every 26 ± 7 d on average. We exposed eight traps per experimental plot in order to overcome the low attractiveness of the chosen color toward Auchenorrhyncha (Rodriguez-Saona et al. 2012). Despite the lower captures compared to yellow traps (Wilson and Shade 1967, Rodriguez-Saona et al. 2012) or sweeping net (Morente et al. 2018), we decided to use the transparent traps in order to avoid attracting individuals from outside the experimental plots and therefore reduce movements of individual between treatments. The attractiveness of yellow traps is based on visual stimuli (Capinera and Walmsley 1978) and can be strongly affected by the structure of the surrounding vegetation (i.e., a trap placed on bare ground is expected to overestimate the catches compared to a trap located amidst higher and denser vegetation). Hence, considering the different ground vegetation height between treatment plots throughout the year, yellow traps could have led to inconsistent results. Similarly, samplings by sweeping net were also excluded due to the different sampling efficiency between each ground cover treatment (tillage with bare ground, frequent mowing, and control with grass of different height) and between ground vegetation and tree canopy (Bodino et al. 2019).

Insect Identification

Captured vectors were individually observed in order to separate them from the Auchenorrhyncha not relevant to the investigation. *Philaenus spumarius*, *P. italosignus*, and *N. campestris* can be distinguished from each other by morphological analysis of both external and sexual characters (Drosopoulos and Asche 1991, Biedermann and Niedringhaus 2009), so we identified the specimens by their external morphology. For species confirmation, a sample of three males for each species from each olive grove was identified by morphological analysis of the genital segments previously macerated in 10% KOH, washed in water and transferred to glycerine. Only *P. spumarius* and *N. campestris* were found.

Statistical Analyses

We explored the effects of soil management (tillage, frequent mowing, and control), trap position (ground vs canopy), sampling date, and their two-way interactions on *P. spumarius* abundance (number of adults per trap, per sampling round) with generalized linear mixed-effects models (GLMM with a Poisson distribution and a log link-function). Within each position and within each sampling round, we pooled the individuals collected by the four traps. All the explanatory variables were included in the models as categorical variables. Since the number of exposure days slightly differed among sampling rounds, this continuous variable was included in the models as an offset. We accounted for the nested design of the study by including site identity (n = 13) as random factor. We performed the analyses using the 'lme4' package (Bates et al. 2017) implemented in R (R Core Team 2020). We checked GLMM models for overdispersion and residual distribution using the 'DHARMa' package (Hartig 2017). There was no evidence of either spatial or temporal autocorrelation of models' residuals (analyses performed using 'ncf' and 'acf' packages, respectively; Bjørnstad 2016). We performed the analysis in R (R Core Team 2020). *Neophilaenus campestris* was not included in the analysis due to the low catches (almost 1 out of 20 traps), the tendency of the adults to migrate on conifer (Mazzoni 2005, Lopes et al. 2014) or remain on grass cover in absence of coniferous plants (Bodino et al. 2020) and its low acquisition and transmission efficiency (Cornara et al. 2016, Cavalieri et al. 2019). In preliminary analyses, we pooled *Philaenus spumarius*

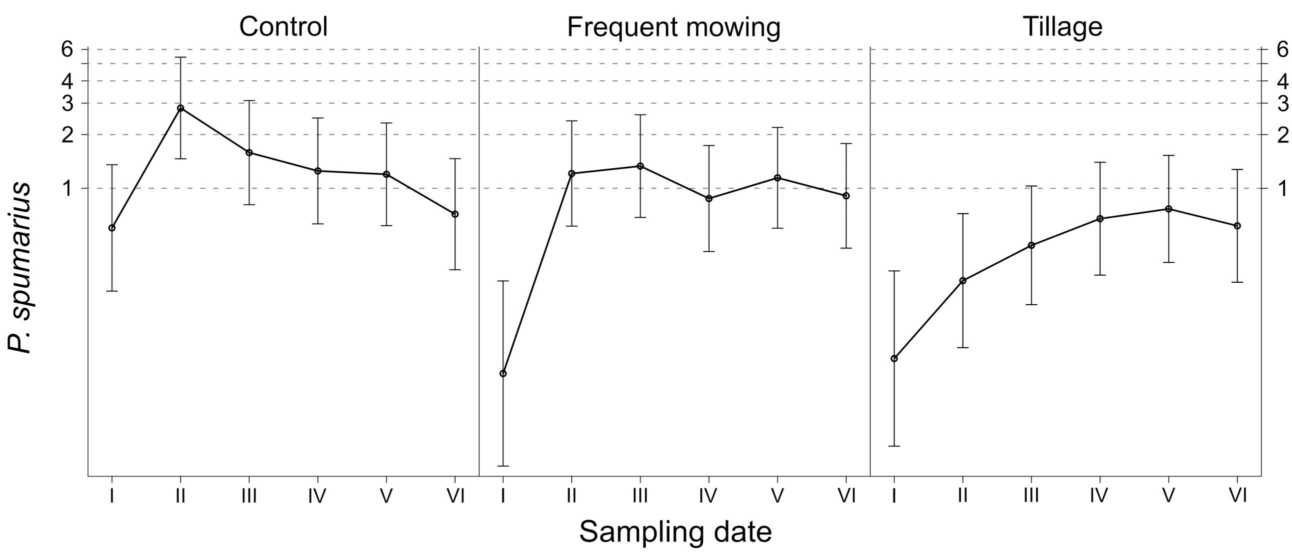

Fig. 2. *Philaenus spumarius* density under control, frequent mowing and tillage plots over the season. Plots were drawn using 'effects' package in R.

and *Neophilaenus campestris* and results were similar to those presented in the main text.

Results

General Results

A total of 829 spittlebugs were captured in 1,768 transparent traps: 727 (mean per trap 0. 42 ± 1.13) were *P. spumarius* and 102 (mean per trap 0. 06 ± 0.28) *N. campestris*. As expected, *P. italosignus* was not found in the study area. The absence of *P. italosignus* was probably due to a lack of suitable hosts (such as *Asphodelus ramosus* (Linnaeus, 1753) (Asparagales: Asphodelaceae)) in the study area (Panzavolta et al. 2019).

Treatment Effects

The cultural practices applied in early spring at *P. spumarius* III-IV juveniles stage prevalence reduced the cumulative captures of adults by 20% in frequent mown plots and by 61% in the tilled ones compared with untreated population observed after the peak of captures (Fig. 2). At the end of the sampling period, no reduction in the vector total density was observed in frequent mown plots. On the contrary, in the tillage plots *P. spumarius* density lower than 49% compared with control plots (Fig. 2). The cultural practice did not influence the canopy colonization behavior, i.e., there was no interaction between trap position and treatment (Table 1), while a significant interaction between sampling date and trap position (ground vs canopy) was observed (Table 1), indicating that *P. spumarius* presence was higher in the ground vegetation in spring and adults moved into the canopy only in late summer (Fig. 3).

Table 1. ANOVA table from the generalized linear mixed-effects model testing the effects of position, sampling round, and treatment on *Philaenus spumarius* adult abundance

Factors	χ^2	df	*P*-value
Trap position	2.0619	1	0.1510
Sampling date	48.2145	5	<0.0001
Treatment	14.8664	2	0.0006
Trap position × Sampling date	29.2471	5	<0.0001
Sampling date × Treatment	50.1258	10	<0.0001
Trap position × Treatment	1.5925	2	0.4510

Discussion

Considering the ecology and behavior of *P. spumarius*, we expected that the early removal of the ground vegetation hosting the juvenile stages either by mowing or tillage should reduce adult vector populations (Buri et al. 2016, Cornara et al. 2018). Our experimental data indicated that both tillage and frequent mowing affected *P. spumarius* populations negatively, but with differences between the two practices. Tillage exhibited the highest control of *P. spumarius* reducing the cumulative density by 61% in spring and still by almost 50% in autumn, while frequent mowing was less effective, reducing *P. spumarius* cumulative density by only 20% in late spring. The stronger effect of tillage was probably due to high direct mortality of juveniles combined with the complete removal of the herbaceous vegetation. Grass mowing in spring, on the other hand, stimulated a quick vegetative regrowth of plants, providing green plant tissues for the re-colonizing adults. It is important to stress that our vegetation management could not be performed in the immediate proximity of the tree trunks, where it was not possible to till and cut the grass due to the olive tree roots. Here, some juveniles could possibly continue the development on the plants that were not affected by the treatment. In addition, in the last sampling date, a higher insect density was reached in the frequent mown plots compared to the control plots. Probably the early spring cuts allowed the establishment of annual plants (Grime 2001, Ilmarinen and Mikola 2009) more attractive for *P. spumarius* and/or the slowdown in growth and leaf production after cutting allowed the maintenance of a greater water content in plants during summer (Biran et al. 1981). In fact, a greener sward was observed in the mown plots compared with control plots.

Regarding the movement of *P. spumarius* between ground vegetation and canopy, it is well known that this species is closely tied to herbaceous plants during its development, in particular to Asteraceae and Fabaceae, while the adults can migrate to trees and shrubs during summer (e.g., Cornara et al. 2018, Bodino et al. 2019). Although previous studies found that vector density on canopies can be very low compared to ground vegetation (Morente et al. 2018), we found low adult abundance on olive trees only in early spring. Bodino et al. (2019) noticed differences in the spatial distribution of the vectors between herbaceous and woody plants during summer according to local climate. The continuous presence of spittlebugs on canopies is probably related to the less severe water stress of olive trees growing in cooler climates compared to warmer and drier

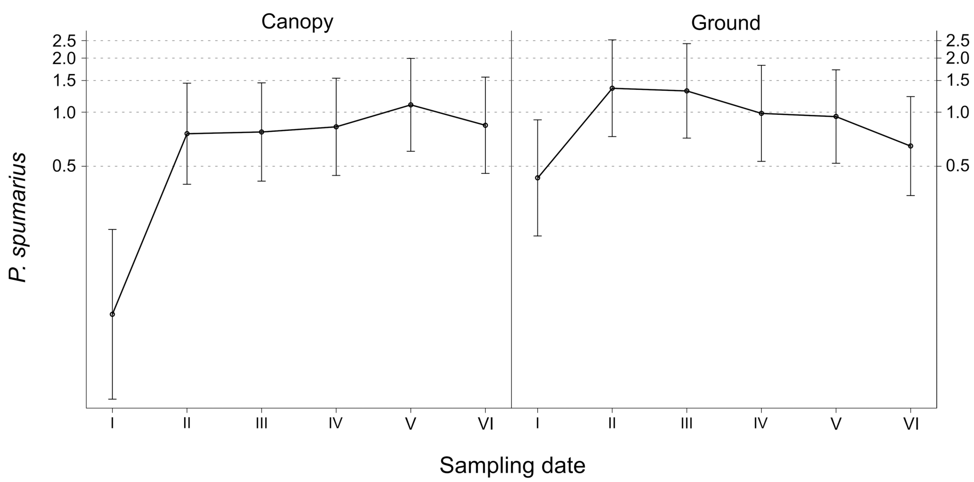

Fig. 3. *Philaenus spumarius* density in canopy and ground vegetation over the season. Plots were drawn using 'effects' package in R.

regions such as Apulia, Spain, and Greece. Indeed, in these countries, adults tend to disappear in summer from the olive trees (Ben Moussa et al. 2016, Tsagkarakis et al. 2018, Antonatos et al. 2019, Bodino et al. 2019). The relatively cooler climate of our investigated area might explain the high density on canopies observed in summer and early autumn.

Neither tillage nor frequent mowing affected the seasonal vertical distribution of *P. spumarius*. The vegetation left around the trunks and the plant tillers remaining after the mowing were probably enough to sustain adults within the olive groves in late spring. The movement seems to be related to a physiological need of the insect to feed on both herbaceous and woody plants, as it is known that also under laboratory conditions, where grasses always actively grow, individuals tend to move on olive trees (EFSA 2019).

In general, when *P. spumarius* is present as juvenile stage, spring tillage caused a reduction in the density of the spittlebug within the olive groves and can be considered a viable option for the containment of *P. spumarius*, while frequent mowing did not greatly reduce the insect population even after four operations throughout the season.

Ground management performed in early season can considerably reduce the juvenile populations, due to their low dispersion capability (Bodino et al. 2020). Consequently, lower populations of adult vectors can develop, limiting the spread of *X. fastidiosa*.

However, tillage can have stronger negative environmental impacts than mowing (Karamahouna et al. 2019) and the ecological consequences of the complete removal of ground cover should be carefully evaluated. The reduction in plant richness caused by tillage can lead to the loss of arthropod biodiversity (Kruess and Tscharntke 2002; Marini et al. 2007, 2009; Rowen et al. 2020) and mobile and generalist herbivores as *P. spumarius* are less likely to be affected by changes in plant composition compared to less mobile and more specialized herbivores (Koricheva et al. 2000). Moreover, tillage in olive groves can cause unsustainable rates of soil loss, especially on steep slopes (Sastre et al. 2017). So, although tillage appeared as a promising control measure for the containment of the vectors possibly reducing the use of agro-chemicals, a careful evaluation of the potential negative impacts of this practice on multiple ecosystem services should be considered.

Acknowledgments

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