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Experimental Nets for a Protection System against the Vectors of *Xylella fastidiosa* Wells et al.

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Abstract: The effectiveness of experimental nets in preventing the access of adult meadow spittlebug *Philaenus spumarius* L., the main vector of *Xylella fastidiosa* Wells et al. subspecies *pauca*, sequence type (ST) 53, in olive tree nurseries and orchards was evaluated. To optimize the net design, mesh size, kind of fabric, thread typology, and radiometric properties, six nets with different mesh sizes and kinds of fabric were evaluated in laboratory and in field experiments. Laboratory bioassays evaluating the capability of adult spittlebugs to pass through nets with different mesh sizes (1.2, 1.8, 2.4 mm) showed that all nets with a mesh size equal to or lower than 2.4 mm prevented insect crossing. These results were confirmed in field conditions using an experimental net box apparatus. Further laboratory tests showed a positive correlation between porosity and radiometric properties of the nets. Three prototypes of thermally stabilized flat woven nets made of circular cross-sectional yarns, knitted net with strips, and knitted nets made of yarns were tested after the evaluation of their potential usability in terms of porosity stability. The knitted net features were found to be the most suitable. The net transmissivity of the total and direct component of solar radiation in the photosynthetically active radiation and the infrared ranges increased with the net porosity. A prism-shaped wooden frame with a triangular base covered with the knitted net with a 2.4 mm mesh confirmed the insect's capability of reaching considerable heights, up to 2.85 m. Hence, based on our results, the monowire knitted net with a 2.4 mm mesh can be used in open field nursery and olive orchards to prevent the access of *P. spumarius* adults and to shield the openings of greenhouse nurseries.

Keywords: insect-proof net; integrated pest management; nursery; olive tree

1. Introduction

In the Apulia region, olive tree cultivation covers almost 370,000 ha, with a yearly economic value of production around 492 M€ [1], representing 53.3% of the overall farmland in the southern area of the region [2]. Moreover, centennial olive trees, which are very common in southern Apulia, are an important attraction for tourists as part of the cultural, traditional, and historical heritage of the region [3].

In 2010, olive trees on the west coast of Salento Peninsula (Apulia region, Italy) began to decline and die with a condition of unknown aetiology called “olive quick decline syndrome” [4]. Saponari et al. [5] demonstrated that olive trees with leaf scorch symptoms (i.e., branches with basal

and apical pale green leaves, later expressing different degrees of scorching starting at the leaf tips, partial defoliation, and death of shoots and branches) were positive for *Xylella fastidiosa* Wells et al. subspecies *pauca*, sequence type (ST) 53 (Xanthomonadales, Xanthomonadaceae) [6]. This study was the first widespread detection of this bacterium in Europe, and a quarantine around the infected area was imposed [7]. Unfortunately, quarantine was not able to limit the diffusion of the bacterium and, by the end of 2013, the affected area had grown to almost the entire Salento Peninsula. *Philaenus spumarius* L. (Hemiptera, Aphrophoridae) is the most common spittlebug species in olive tree orchards in the Salento Peninsula, and it has been reported as the main vector of *X. fastidiosa* [5,8].

The nursery industry is an important part of the agricultural sector in Apulia, and the production of “*Xylella*-free” plants is therefore of strategic importance for local and foreign markets [9]. In fact, *X. fastidiosa* affects not only olive trees but also more than 30 different commercial crops and wild species [10]. In this context, the development of preventive control measures to avoid bacterium transmission by known and potential insect vectors is crucial, as is the selection of tolerant or resistant olive tree varieties [11].

In recent years, the use of insect-proof nets in protected cultivation has increased, mainly to reduce the use of pesticides [12–14]. Due to the small size of some insect pests (i.e., thrips, aphids), very fine mesh nets are required [13,15]. Consequently, the use of such nets significantly reduces greenhouse ventilation [16,17] and modifies the exchange of radiation and mass between crops and the atmosphere [12,18]. In some cases, microclimate modifications induced by insect nets can improve yield in addition to reducing pesticides [19,20].

The mesh of the net should be as wide as possible in order to minimize the effects on environmental parameters (temperature, air humidity, solar radiation transmission, permeability to air) and structural issues (visual impact, wind loads, size of supporting structures) [13]. The size of the mesh also influences the distribution of flying insects near windbreak fences [21].

X. fastidiosa transmission by *P. spumarius* is mainly attributed to the adults, which are able to fly; juveniles do not fly and live on herbaceous plants protected in the spittle they produce [22]. Moreover, the low chance that young instars might transmit the bacterium is further reduced by the loss of infectivity with the moult [8]. Therefore, in order to successfully protect olive trees, the mesh of the net should prevent the passing through of *P. spumarius* adults [5,23].

In the present study, laboratory and semi-field behavioral tests were carried out in order to evaluate the capability of experimental nets with different mesh sizes and textures to prevent the passing through of *P. spumarius* adults. Based on the laboratory and semi-field tests, the net with the wider mesh size and the most suitable fabric for the purpose was identified. Moreover, the main radiometric properties of the experimental nets were determined. Finally, a demonstration trial was undertaken to preliminarily evaluate in the field the vertical distribution of *P. spumarius* adults in the first four meters above the ground level.

2. Materials and Methods

Three experimental nets with different mesh sizes ($d_1 = 1.2$ mm, $d_2 = 1.8$ mm, $d_3 = 2.4$ mm), selected on the basis of adult *P. spumarius* body length (male, 5.3–6.0 mm; female 5.4–6.9 mm) and head width (2.5 mm) [24], were tested. In this study, the mesh size was defined as the maximum dimension of a line segment inscribed in the hole of the net.

Three prototypes of different textures were chosen (Figure 1): (1) a thermally stabilized flat woven net (F) with transparent yarns; (2) knitted net made of white strips and transparent yarns (KS); (3) knitted net only made of transparent yarns (KW). Monofilaments had a circular cross section and were made of high-density polyethylene (HDPE); strips were made of low-density polyethylene (LDPE). Due to manufacturing limitations, six nets were tested: F- d_1 flat woven with mesh size 1.2 mm, F- d_2 flat woven with mesh size 1.8 mm, F- d_3 flat woven with mesh size 2.4 mm, KS- d_1 knitted net made of strips and wires with mesh size 1.2 mm, KS- d_2 knitted net made of strips and wires with mesh size 1.8 mm, and KW- d_3 knitted net only made of wires with mesh size 2.4 mm.

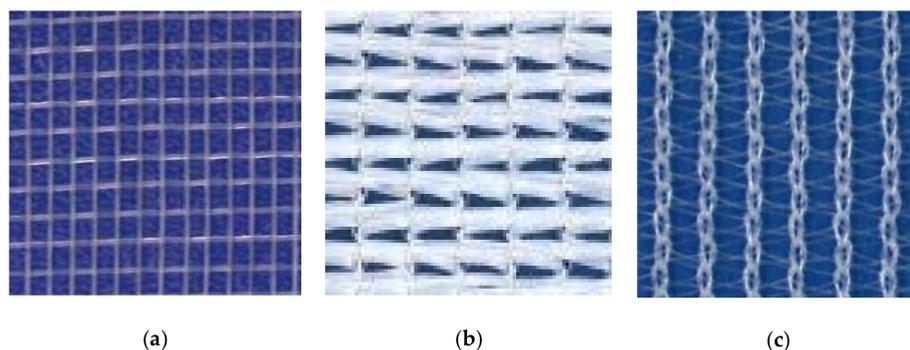


Figure 1. Pictures of the nets tested: (a) F-d₃, flat woven with mesh size 2.4 mm; (b) KS-d₂, knitted net made of strips and wires with mesh size 1.8 mm; (c) KW-d₃, knitted net made only of wires with mesh size 2.4 mm.

Experimental nets were manufactured by SACHIM SRL (Putignano, Bari, Italy).

2.1. Laboratory Behavioral Bioassays

Nymphs of *P. spumarius* were collected from spontaneous herbaceous vegetation (mainly, the genera *Sonchus*, *Foeniculum*, *Medicago*, *Vicia*) near Foggia (Apulia, Italy) and transferred to shoots of *Vicia faba* L. seedlings in plexiglas cages and maintained at 23 ± 2 °C, $70 \pm 5\%$ relative humidity (r.h.), and 14:10 light:dark (L:D) photoperiod. Emerged adults were daily transferred to new cages and provided with fresh *V. faba* seedlings. Adults that were 1–2 days old were used for the experiments.

In order to test the capability of adult spittlebugs to move through different experimental nets, laboratory bioassays were carried out. The device used was made of two glass cylindrical jars separated by the net to be tested. Ten insects were released in the upper jar (\varnothing 25 cm \times 20 cm), whereas two-week-old faba bean plants ($n = 5$) were positioned in the lower jar (\varnothing 25 cm \times 20 cm) with the apical shoots at least 3 cm below the net to avoid insect feeding. The upper jar was closed with nonwoven fabric to prevent insect escape. Insects were kept without food to motivate them to reach the shoots underneath. The number of insects crossing through the net was measured at 24-h intervals until all insects were dead. For each net, six replicates were performed.

2.2. Semi-Field Behavioral Tests

The experimental nets were further evaluated in semi-field tests using an experimental apparatus (net box). A young plant of olive tree was put inside a steel frame (40 \times 40 cm base, 80 cm height) covered with one of the experimental nets. The frame with the plant was placed in a larger one (80 \times 80 cm base, 120 cm height) covered with an anti-aphid net, where 25 *P. spumarius* adults were released (Figure 2). Because no water or food was supplied to the insects, they were expected to be strongly motivated to pass through the net and reach the olive tree in the inner box. For each type of net, four replicates were performed. Tests were carried out during April and May 2017 at the experimental station of the University of Bari (Valenzano, BA). The presence of insects in the inner box with the plant was checked every 24 h until all insects were dead.

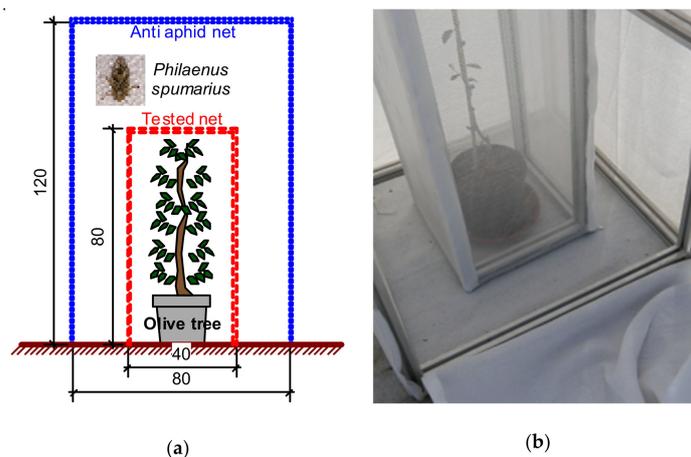


Figure 2. Net box used for field test: (a) construction scheme and (b) picture. Units are in cm; the image of the insect in the construction scheme is not to scale.

2.3. Radiometric Tests

Radiometric tests on the nets were carried out at the University of Bari by means of spectrophotometer (1760X, Perkin-Elmer Instruments, Norwalk, CT, USA). The spectral transmissivity $\tau(\lambda)$ was evaluated over different wavelength bands: solar and near-infrared radiation wavelength range (290–2500 nm), photosynthetically active radiation (PAR) range (400–700 nm), and far-infrared radiation (long-wave infrared, LWIR) range (>2500 nm). Spectral direct perpendicular transmissivity was measured in the solar and near-infrared radiation range (290–2500 nm in steps of 10 nm) by a double beam with a direct perpendicular incidence UV-VIS-NIR spectrophotometer (Lambda 950, Perkin Elmer Instruments, Waltham, MA, USA). Spectral transmissivity in the LWIR range, between 2500 and 25000 nm, was measured by a FT-IR spectrophotometer using radiation with a direct perpendicular incidence. The transmissivity coefficients in the LWIR range were calculated as average values of the spectral transmissivity in the wavelength range from 7500 to 12500 nm. For each experimental net, five samples were tested; the average values and standard deviation of radiometric properties were calculated.

The porosity ($\Phi = Se/St$) is defined as the ratio between the surface of empties (Se) and the area of the net (St). It is one of the main parameters involved in the radiometric properties of the net [25,26]. Porosity plays an important role in the evaluation of the air permeability of the net and, consequently, of wind loads on the structure [27]. The porosity of tested nets was measured by means of image analysis [25].

2.4. Field Demonstration Trial

To preliminarily evaluate the vertical distribution of *P. spumarius* adults in the first four meters above the ground level, a prism-shaped wooden frame (4.0 m high) with a triangular base (1.0 m \times 1.0 m \times 1.0 m) was covered with a KW-knitted net (2.4 mm mesh) (Figure 3). On the three sides of the frame, 12 yellow sticky traps (20 \times 20 cm) (Novapher, Milan, Italy) were placed [28] at different heights (40, 85, 185, 285, 390 cm). The triangular shape of the net-covered frame was designed in order to evaluate the incidence of adult spittlebugs on the net for any direction. The frame was installed in an olive tree orchard located at the experimental station of the University of Bari (41°01'16.48" N, 16°54'18.39" E, Elev. 120 m) from July 3 to October 31, 2017. Traps were replaced four times (31 July, 31 August, 30 September, 31 October), and spittlebugs stuck on the traps were identified and counted.



Figure 3. Wooden frame covered with the net and yellow sticky traps at different heights.

3. Results

In laboratory and in semi-field experiments, none of the *P. spumarius* adults was able to pass through the experimental nets with different textures and a mesh size range of 1.2–2.4 mm. Therefore, a net with mesh size equal to or lower than 2.4 mm can successfully prevent the arrival of meadow spittlebugs on plants and, hence, the possible transmission of *X. fastidiosa*.

The analyzed nets showed potentially different stability in terms of hole shapes and mesh dimension. The prototype of flat woven nets could be subject to damages of some thermal connections. Knitted net with strips showed trip twisting and a very low porosity. Knitted nets with wires very hardly showed lack of stability or twisting with consequent variation of porosity (Table 1).

Table 1. Construction characteristics of nets tested. Flat woven net (F), knitted nets made of strips and yarns (KS), and only of yarns (KW); d is the maximum linear dimension of the mesh (mm).

Tested Net	d (mm)	Porosity (%)	Warp Diameter (mm)	Weft Diameter (mm)
F-d1	1.2	58.11	0.28	0.28
F-d2	1.8	64.62	0.28	0.28
F-d3	2.4	71.60	0.28	0.28
KS-d1	1.2	6.27	0.26	Strips (90 μm thickness)
KS-d2	1.8	18.00	0.26	Strips (60 μm thickness)
KW-d3	2.4	42.96	0.23	0.23

Laboratory tests (Table 2) confirmed the correlation between porosity and radiometric properties of the nets tested. The transmissivity of total ($\tau\text{-Tot}$) and direct component of solar radiation in PAR range ($\tau\text{-Dir}$) increased with the porosity as well as the transmissivity of the net in the IR range. As a result, the greenhouse effect was minimized with a higher porosity of the net (Figure 4). Haze and transmissivity of the diffuse component of the solar radiation in PAR ($\tau\text{-Dif}$) showed, as expected, an opposite behavior, i.e., both decreased as the porosity increased (Figure 4).

In field demonstration trial, all yellow sticky traps captured eight and nineteens adults of *P. spumarius* and *Neophilaenus campestris* (Fallen), respectively. For the latter species, the role of vector in the transmission of *X. fastidiosa* was recently demonstrated (unpublished data). Vertical distribution of insect vector captures was almost uniform in the first 285 cm above ground level, while no insect catches were found from 285 to 390 cm. Considering the low number of insects captured and their uniform distribution in the first 285 cm above the ground level, statistical analysis was not performed.

Table 2. Radiometric properties of the nets tested (%). τ -Tot total transmissivity in photosynthetically active radiation (PAR) (400–700 nm), τ -Dir direct transmissivity in PAR range, τ -Dif diffuse transmissivity in PAR range, Haze in PAR range, τ -LWIR transmissivity in far infrared (7500–12500 nm). Data are expressed as measured average values \pm values of standard deviation.

Tested Net	τ -Tot	τ -Dir	τ -Dif	Haze	τ -LWIR
F-d1	0.967 \pm 0.010	0.585 \pm 0.006	0.383 \pm 0.004	0.396 \pm 0.005	0.553 \pm 0.011
F-d2	0.975 \pm 0.013	0.650 \pm 0.010	0.325 \pm 0.004	0.333 \pm 0.010	0.617 \pm 0.011
F-d3	0.979 \pm 0.012	0.703 \pm 0.009	0.276 \pm 0.005	0.282 \pm 0.012	0.690 \pm 0.013
KS-d1	0.511 \pm 0.021	0.047 \pm 0.003	0.464 \pm 0.019	0.908 \pm 0.016	0.348 \pm 0.008
KS-d2	0.545 \pm 0.023	0.229 \pm 0.010	0.316 \pm 0.012	0.579 \pm 0.011	0.398 \pm 0.016
KW-d3	0.948 \pm 0.016	0.531 \pm 0.009	0.417 \pm 0.007	0.440 \pm 0.009	0.488 \pm 0.012

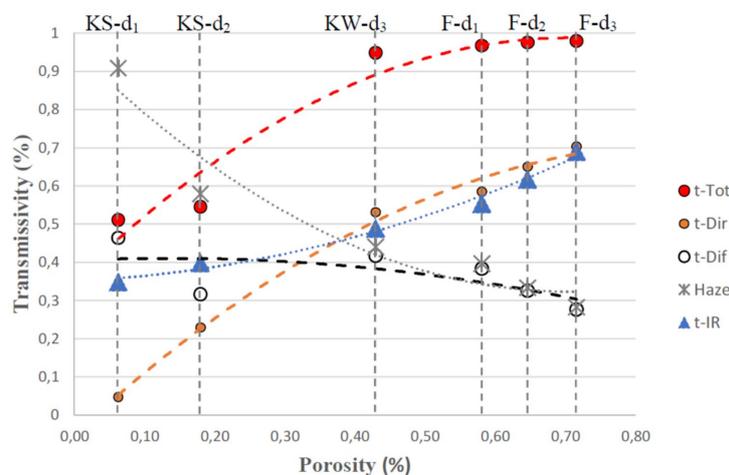


Figure 4. Correlation between average values of radiometric properties of the nets tested and Φ , porosity. Dashed lines represent polynomial correlation curves of measured parameters.

4. Discussion

P. spumarius adults, unable to reach the vegetation, died within 24 and 48 h after the experiments started in semi-field and laboratory conditions, respectively. This is consistent with the observations made by Weaver and King [29], who reported that succulent foliage suits the adults the best as it offers a plentiful supply of moisture.

Laboratory and semi-field experiments showed that a net with the mesh size equal to or lower than 2.4 mm can successfully prevent the arrival of meadow spittlebugs on plants and, hence, the possible transmission of *X. fastidiosa*. Concerning the stability of the mesh size, the prototype of flat woven net showed some damages to the yarn thermal connections. For this reason, the prototype of flat woven did not fit our purpose; therefore, the knitted net features represent the most suitable fabric.

Moreover, nets with the highest porosity minimize the visual impact in the field and reduce the wind loads on the supporting structures. In addition, its permeability to the air reduces the impact on environmental parameters crucial for plant growth (i.e., temperature, air humidity, transmission of the solar radiation). Consequently, among the nets tested, those with the higher mesh size ($d_3 = 2.4$ mm) would reduce the impact on the net supporting structure and on cultivation.

With regard to radiometric properties, the high transmissivity values achieved for F-d1, F-d2, and F-d3 nets were due to a combination of high porosity and transparent wires (Table 2). It is interesting to note that the τ -Tot measured for F-d1, F-d2, and F-d3 nets was almost constant, while the other radiometric properties showed a variation with the porosity (Table 2). At higher porosity levels, the size of holes prevailed on the radiometric characteristics of the material the threads were made of, and a couple of values, τ -Dif/ τ -LWIR and Haze/ τ -Dif, were almost coincident (Table 2) [30].

Preliminary results of field tests showed that the maximum height at which adult spittlebugs were trapped (2.85 m) was likely lower than what they can actually reach. In fact, in a previous study, Weaver and King [29], found that 60 *P. spumarius* adults, out of 1470 captured on tangle-foot bands placed at various heights, reached 5.90 m above ground. Considering the proven capability of vectors to reach considerable heights, either by flying or being transported by wind, the net should entirely cover the plants in open field nursery and olive orchards or should be designed with appropriate height to be defined in future studies. In protected cultivation, the net should be installed to shield the openings of greenhouse nurseries from the entrance of the *X. fastidiosa* insect vectors.

5. Conclusions

All tested nets avoided any intrusion of meadow spittlebug adults, and plants were successfully protected. The monofilament knitted experimental net with the widest mesh ($d_3 = 2.4$ mm, KW-d3) did not show any damage or twisting and variation of the porosity. Therefore, among the tested prototypes, KW-d3 would minimize the visual impact in the field and reduce both the wind loads on the supporting structures and the impact on environmental parameters. Laboratory tests confirmed the correlation between porosity and radiometric properties of the nets tested. The transmissivity of total ($\tau\text{-Tot}$) and direct component of solar radiation in PAR range ($\tau\text{-Dir}$) increased with the porosity as well as the transmissivity of the net in the IR range.

In open field nursery and olive orchards, the net should entirely cover the plant or be designed with appropriate height to be defined in future studies.

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