Diminished UV radiation reduces the spread and population density of *Macrosiphum euphorbiae* (Thomas) [Hemiptera: Aphididae] in lettuce crops

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Abstract

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UV-absorbing covers reduce the incidence of injurious insect pests and viruses in protected crops. In the present study, the effect of a UV-absorbing net (Bionet) on the spatio-temporal dynamics of the potato aphid on lettuce plants was evaluated. A field experiment was conducted during three seasons in two identical tunnels divided in four plots. A set of lettuce plants were artificially infested with *Macrosiphum euphorbiae* adults and the population was estimated by counting aphids on every plant over 7 to 9 weeks. Insect population grew exponentially but a significantly lower aphid density was present on plants grown under the UV-absorbing cover compared to a standard 50 mesh net. Similarly, in laboratory conditions, life table parameters were significantly reduced under the Bionet. Moreover, SADIE analysis showed that the spatial distribution of aphids was effectively limited under the UV-absorbing nets. Our results indicate that UV-absorbing nets should be considered as an important component of lettuce indoor cropping systems preventing pesticide applications and reducing the risk of spread of aphid-borne virus diseases.

Keywords: potato aphid; UV-absorbing net; IPM; SADIE; life table parameters

Integrated Pest Management (IPM) involves the use of multiple tactics to optimize the control of all classes of pests by reducing pesticide use, providing economic savings for the farmer, and protecting both the environment and human health (Ehler 2006). Aphids are among the most damaging insects of outdoor and indoor lettuce causing significant losses (Parker et al. 2002; Nebreda et al. 2004). Among the control tactics that should be developed in IPM, the use of physical or mechanical methods are included, which avoid residues on commodities and minimize damage to the environment (Weintraub, Berlinger 2004). UV-absorbing covers were proven to reduce the impact of several insect pests such as aphids, whiteflies, moths and thrips (Diaz, Fereres 2007). In fact, UV radiation is important in the visual cues of most insects, modifying their flying and alighting behavior, thus,

governing its dispersal activity (Raviv, Antignus 2004).

In the case of aphids, not only an effect in orientation was described but also a reduced colonization was detected in pepper (Chyzik et al. 2003) and in lettuce (Diaz, Fereres 2007). Although the market presently offers different types of photoselective materials, the impact of covers that filter radiation in the UV spectrum (280–400 nm) on aphid population growth and dispersal activity are weakly known. This would provide useful information because aphid population dynamics (Dixon 1988; Kindlmann et al. 2007) and dispersal are the main topics of study aiming at controlling these species. *M. euphorbiae* is one of the most harmful aphid species worldwide (Blackman, Eastop 2007), colonizing lettuce (Nebreda et al. 2004) and transmitting more than 40 non-persistent viruses (Blackman, Eastop 2007). For these reasons, *M. euphorbiae* was selected in this study with the purpose of describing the spatio-temporal dynamics of aphids in a lettuce crop grown under UV-blocking nets.

MATERIAL AND METHODS

Experimental field design

The experiments were conducted in "La Poveda experimental farm", Arganda del Rey, Madrid, Spain (40°18'N, 3°26'W), using two identical "tunnel type" greenhouses (6.5 m wide, 8 m long and 2.6 m height) covered by each of the two types of nets to be tested, following a similar experimental set-up as described by Diaz et al. (2006). We focused our study on aphid population dynamics over time (7 to 9 weeks) in the lettuce crop under the two types of covers. Experiments were repeated in three separate lettuce-growing cycles (autumn 2007 and spring 2008, 2009). Greenhouses were identical, separated five meters and divided in four equal plots separated with vertical walls made from regular 50-mesh nets. The nets to be tested had similar physical properties (mesh size, open area, mechanical strength) except for their differences in absorbing UV radiation. Bionet (Meteor Agricultural Nets, Ltd., Petach-Tickva, Israel) filtered radiation in the UV spectrum; while the standard 50 mesh (Criado y López, El Ejido, Spain) had no special UV-absorbing properties and was used as control. The radiation transmitted inside the tunnels was measured several sunny days in each plot at midday using a portable quantum meter

radiometer (UVM, Apogee, Logan, USA), sensitive to ultraviolet radiation (UV), 320–400 nm.

In each plot, 64–66 lettuce plants (*Lactuca sativa* L.) were transplanted in early September during 2007 and in the beginning of March during 2008 and 2009, spaced 0.3 m along water drip rows and 0.5 m between the eleven rows within each plot. Lettuce seedlings obtained from a local nursery were pest-free and transplanted at the 4-leaf stage. Two romaine cultivars; Aitana (Ramiro Arnedo, Calahorra, Spain) and Moratina (Syngenta Seeds, Barcelona, Spain), were used and the growing season was completed in 54 to 69 days after the transplant, when the lettuce plants had fully developed their commercial head and were harvested. In the spring 2008, temperature under the covers was monitored by Tinytag data loggers (Gemini Data Loggers, Chichester, UK) and meteorological data were provided by a weather station located close to the farm.

One week after transplanting, a group of lettuce plants in rows one and two were artificially infested with 33 winged adults of *M. euphorbiae,* placing them softly on the leaves using a fine paintbrush to simulate a natural aphid infestation into a lettuce indoor crop and starting the experiment with a density of 0.5 aphids per plant in each plot. The aphid colony, which was started from a single virginoparous apterae collected in Villa del Prado in Spain on lettuce in 1999, were reared in a climatic chamber (16L:8D, 23–18°C, 60–70% RH) on lettuce plants (*L. sativa* cv. Moratina).

Aphid sampling procedures

Aphid sampling was conducted every week counting *in situ* the density of aphids on every plant grouped in categories of infestation 0 to 5. For the purpose of regression analysis, the following numerical values or counts within each category were assumed for the various classes (0:0 aphids; 1:3 aphids; 2:12 aphids; 3:34 aphids; 4:100 aphids; 5:150 aphids) and the average density found in each plot was considered the response variable. Plant phenology was weekly described using the extended BBCH scale (Meier 1997).

Spatial analysis of aphid distribution

The spatial distribution of aphids in each lettuce plot was studied by means of the Spatial Analysis

by Distance IndicEs (SADIE) method. The Index of aggregation (I_a) considers the spatial distribution as either random ($I_a = 1$) or aggregated ($I_a > 1$). Furthermore, the SADIE technique provides indices that measure the extent to which every unit contributes to the global clustering; the Index of clustering in patches ($v_i > 1.5$) and gaps ($v_j < -1.5$) that can be mapped in "red-blue" plots (Perry et al. 1999). The spatial analysis was restricted to rows 3 to 11 that were free of aphids at the beginning of the experiment, thus, exposed to secondary spread of aphids moving from the infested source plants (rows 1 and 2).

Statistical analysis

Statistical analysis was performed with the IBM Statistics SPSS v.17.0 package for windows (IBM Co., NY, USA). Data were fitted into temporal models for every season and type of net. The density of insects fitted a growth model, in which time was considered the "independent variable" of the regression analysis. Repeated Measures GLM was selected to study the main effects: type of net and time (Vonesh, Chinchilli 1997). A repeated factor (time), a main fixed factor (type of net) and a covariate (UV radiation transmitted in each plot), were defined in the model. The density of insects was log-transformed to achieve linearity prior to apply GLM. To observe any differences at the end of the crop, comparisons between the types of net were performed using a two samples student *t*- test ($P \leq 0.05$).

Life table parameters of *M. euphorbiae*

To compare the *M. euphorbiae* life table parameters under different UV environments, ten lettuce plants (*L. sativa* cv. Aitana) at growth stage 16 (BBCH) were grown in a glasshouse inside cages (0.6 m wide × 0.8 m long × 0.6 m tall) and covered by each of the two types of nets (Bionet or standard 50 mesh). Temperature and relative humidity were kept almost constant (15 ± 2°C; 70 ± 10%). To improve the lighting conditions inside the glasshouse, one lamp Ultra-Vitalux (Osram, Munich, Germany) emitting a sun-like radiation spectrum was placed over each cage. The protocol to determine the life table parameters of *M. euphorbiae* under each type of net was similar to that described by Fereres (1989), placing a single winged adult

aphid in 10 independent clip-cages for a period of 24 hours. The nymphs born in that period were used to calculate the parameters: pre-reproductive period (*d*), effective fecundity (M_d), intrinsic rate of natural increase (r_m) and mean relative growth rate (MRG). A student *t*-test ($P \leq 0.05$), was used to compare the parameters under the two types of nets.

RESULTS AND DISCUSSION

UV, temperature and light properties

A higher amount of UV radiation (22–30%) was transmitted under the standard net compared to the photoselective cover, Bionet. Moreover, no differences in UV radiation were found among the plots covered with the same type of net (Table 1). Temperature in the first two weeks of the autumn experiment ($T_{mean} = 20.4 \pm 0.6$°C) was optimal for insect development; whereas in spring, to a greater extent in 2008, minimum temperatures below aphid developmental thresholds were often recorded ($T_{min\ 2008} = 2.1 \pm 1.1$°C, $T_{min\ 2009} = 3.8 \pm 0.6$°C). In addition, maximum temperature increase in the tunnels comparing to the outside records was significantly different ($t = -7.8$, $df = 120$, $P < 0.001$) between the two nets: Standard (10.2 ± 2.3°C) and Bionet (7.3 ± 1.7°C). The lower mean temperature recorded under Bionet may have delayed aphid development (Dixon 1988), reducing insect density.

Temporal dynamics of aphid populations

In all three seasons studied, the density of *M. euphorbiae* was always greater under the standard net than under the UV-absorbing cover (autumn 2007: $F = 20.677$, $P < 0.001$; spring 2008: $F = 5.082$, $P = 0.042$; and spring 2009: $F = 8.807$, $P = 0.010$) (Fig. 1). The

Table 1. Percentage of transmitted UV radiation monitored during the experiments (mean ± SE)

	Bionet	Standard
Autumn 2007	21 ± 1[a]	43 ± 1[b]
Spring 2008	27 ± 2[a]	57 ± 3[b]
Spring 2009	17 ± 1[a]	43 ± 1[b]

Different letters between Bionet and Standard refer to significant differences in the student *t*-test ($P < 0.05$)
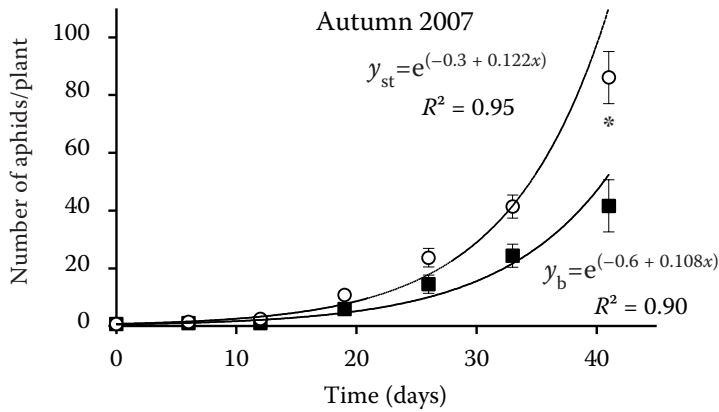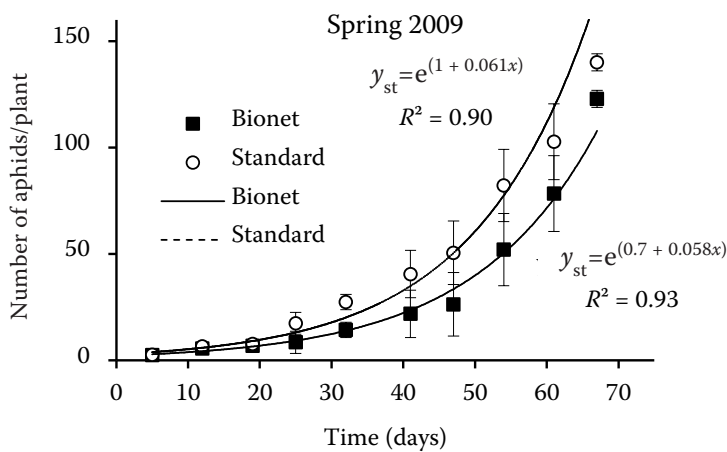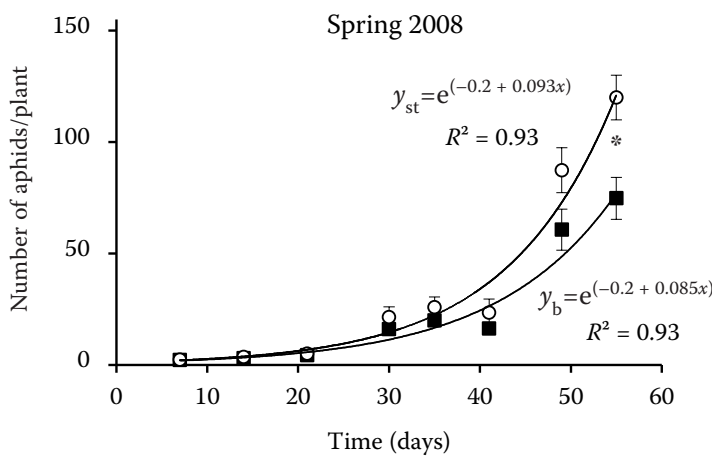
Fig. 1. Growth of *M. euphorbiae* population in the plots covered with a UV-absorbing net (Bionet: ■ ——) and a Standard net (○ - - - -). The growth model is shown by the equation [$y = \exp(b_0 + b_1 t)$] and the coefficient of determination (R^2). An asterisk (*) shows differences between the nets at harvest

time effect was highly significant in the models during the three seasons ($P < 0.001$); whereas the GLM only indicated significant interaction between the two factors (type of net and time) in autumn 2007 ($F = 5.473$, $P = 0.005$). As observed in Fig. 1, aphid density at harvest was greater under the standard than under the photoselective cover in autumn 2007 ($t = -2.906$, $df = 6$, $P = 0.027$) and spring 2008 ($t = -3.282$, $df = 6$, $P = 0.017$). Benefits derived from this result include the reduction in chemical treatments that would be necessary to obtain a similar percentage of pest-free marketable plants. The population of *M. euphorbiae* displayed an exponential growth curve as shown by high R^2 values (Fig. 1), and the magnitude of the rate of increase (b_1) was lower under the photoselective cover than under the standard net in all seasons. The

exponential curve reflected the progressive increase of aphids in parthenogenetic reproduction without resource limitations, which can frequently occur in spring and autumn (KINDLMANN et al. 2007). Coefficient values in the exponential equations were greater in autumn than in spring. This may be due to the low temperatures recorded in spring at the beginning of the experiment, below zero degrees in 2008, which may have delayed aphid development time.

There were significant differences in the development of *M. euphorbiae* under the two types of nets for the following life table parameters: pre-reproductive period (Bionet: 14.6 ± 0.5, standard: 13.5 ± 0.3; $t = 5.400$, $df = 14$, $P < 0.001$), intrinsic rate of increase (Bionet: 0.13 ± 0.01, standard: 0.17 ± 0.01; $t = -2.515$, $df = 14$, $P = 0.025$) and mean relative growth rate (Bionet: 0.15 ± 0.01, standard: 0.19 ± 0.01; $t = -2.519$, $df = 14$, $P = 0.025$). The results overall indicated that aphid population growth was significantly reduced under the photoselective nets.

Aphid density was consistently lower under the Bionet than the standard 50 mesh net, which substantiates the results obtained previously on the effect of photoselective covers on the widespread and generalist aphid *Myzus persicae* (Sulzer), feeding on pepper crops (CHYZIK et al. 2003). It is known that differences in environmental conditions created under each type of cover (mainly temperature and light quality) are the key extrinsic factors that affect aphid population development (DIXON 1988). Moreover, the reduction in the amount of

UV radiation that penetrated through the net may also have influenced plant physiology because the lack of UV radiation may have reduced acclimation and repair responses in plants. In addition, this may have modified secondary metabolism or have altered the production of phytohormones (JANSEN et al. 1998). Thus, it is likely that aphids were exposed to a different composition of sap when feeding on lettuce plants covered with Bionet, which could had affected negatively its development. As a result, there could be both, direct and indirect effects, on aphids mediated by their host plants when grown under photoselective covers.

Spatial distribution

Values associated with the Index of aggregation (I_a) were above 1.5 once the pest population density increased regardless of the type of net used. Therefore, according to the SADIE analysis, aphid distribution was aggregated (Table 2). The GLM analysis comparing the Index of aggregation between the two types of nets was not significant in the interaction term ($P > 0.05$); whereas the time factor was significantly different in all seasons ($P < 0.05$). Furthermore, the spread of the aphid population along the plots occurred to a larger extent in the standard cover, or in other words, our results showed that aphid spread was reduced under the UV-absorbing cover. This was indicated by the mean values of I_a, which were consistently lower for most of the sampling dates under

Table 2. Comparison of Index of aggregation (I_a) for the two types of net studied (mean ± SE)

		Autumn 2007 (day)								
		6	12	19	26	33	41			
ns[1]	Bionet	1.2 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	2.3 ± 0.2	2.1 ± 0.1	2.1 ± 0.2			
	Standard	1.2 ± 0.1	1.1 ± 0.3	1.3 ± 0.3	1.6 ± 0.3	2.0 ± 0.2	2.1 ± 0.1			
		Spring 2008 (day)								
		7	14	21	30	35	41	49	55	
ns[1]	Bionet	1.5 ± 0.3	1.8 ± 0.5	1.8 ± 0.6	2.2 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	2.0 ± 0.3	2.5 ± 0.3	
	Standard	1.4 ± 0.1	1.5 ± 0.2	1.8 ± 0.3	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	2.1 ± 0.1	1.8 ± 0.2	
		Spring 2009 (day)								
		5	12	19	25	32	41	47	54	61	67
*[1]	Bionet	1.9 ± 0.1	2.3 ± 0.2	2.6 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.2 ± 0.2	2.0 ± 0.3	2.2 ± 0.2	3.2 ± 0.3	3.3 ± 0.4
	Standard	1.7 ± 0.2	2.1 ± 0.3	2.2 ± 0.4	2.4 ± 0.3	2.2 ± 0.3	2.8 ± 0.3	2.6 ± 0.2	2.9 ± 0.4	2.2 ± 0.6	1.9 ± 0.3

Index of aggregation values of four plots per type of net (Bionet or Standard), were averaged in the table ($n = 4$). When I_a was greater than 1.5, the aphid population was considered aggregated. [1]an asterisk show statistical differences found between the two nets (GLM, $P < 0.05$)

Standard 50 mesh   **Bionet**

$Ia =$	1.3; NS	2.3; **	1.4; NS	2.1; **		$Ia =$	2.1; **	2.1; **	2.1; **	2.7; **
$Vi =$	1.2; NS	1.9; **	1.4; NS	1.9; **		$Vi =$	2.0; **	2.1; **	2.0; **	2.7; **
$Vj =$	-1.1; NS	-2.1; **	-1.3; NS	-1.8; **		$Vj =$	-2.1; **	-2.2; **	-2.0; **	-2.7; **

Day 29, BBCH 39

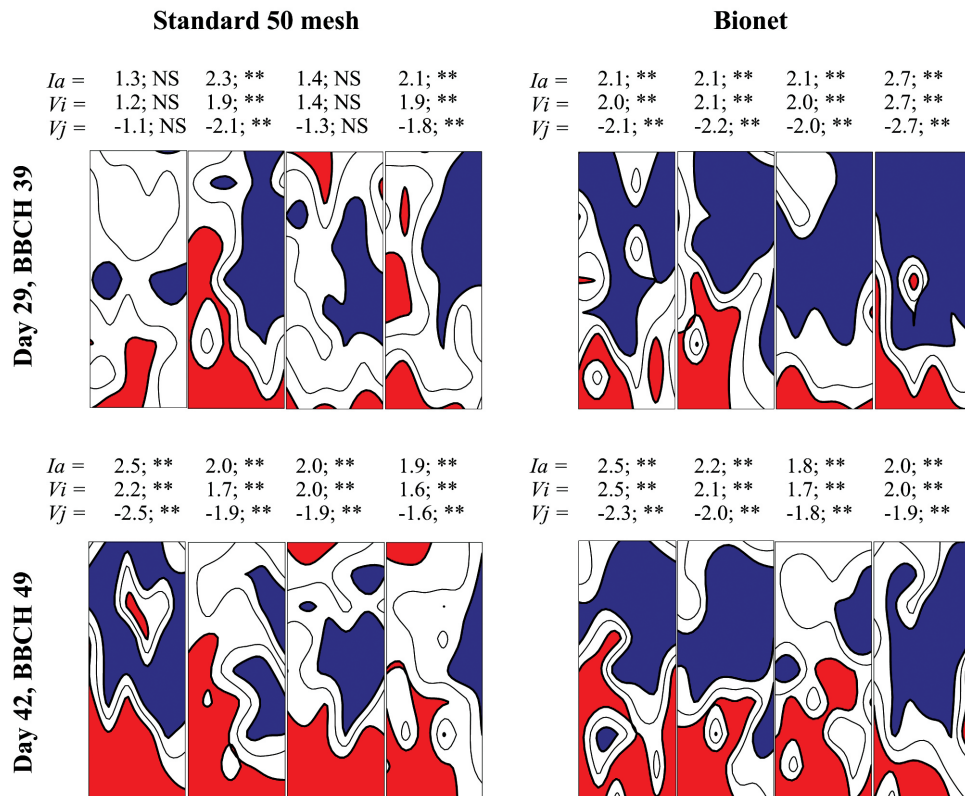|									
$Ia =$	2.5; **	2.0; **	2.0; **	1.9; **		$Ia =$	2.5; **	2.2; **	1.8; **	2.0; **
$Vi =$	2.2; **	1.7; **	2.0; **	1.6; **		$Vi =$	2.5; **	2.1; **	1.7; **	2.0; **
$Vj =$	-2.5; **	-1.9; **	-1.9; **	-1.6; **		$Vj =$	-2.3; **	-2.0; **	-1.8; **	-1.9; **

Day 42, BBCH 49

Fig. 2. Spatial distribution of *Macrosiphum euphorbiae* in autumn 2007 is shown using SADIE methodology in every plot for the BBCH phenological stages: 39 (beginning to form the marketable head) and 49 (fully developed marketable head). Individual values for the Index of clustering (v_i and v_j) allow to represent patches in red ($v_i \geq 1.5$) and gaps in blue ($v_j \leq -1.5$). For each map, the global value of I_a, v_i and v_j are followed by *($P < 0.05$) **($P < 0.01$) or NS ($P > 0.05$)

the standard net. Aphid colonisation process is highly related to insect density because one of the main extrinsic factors that induce aphid dispersion is overcrowding, that leads to search for new feeding sources (Irwin et al. 2007) and this fact would explain why dispersion was lower under the UV-absorbing nets.

Once the clustering indices are plotted using SADIE, the exact location of the foci can be visually determined (Fig. 2). Aphid patches (drawn in red areas) were widely spread along the plot in the standard cover whereas under the Bionet they appeared close to the side where aphids were initially released, at the bottom edge of each map. These results are in line with those reported by Raviv and Antignus (2004) stating that aphids tend to disperse less under UV-deficient environments due to an alteration of their visual cues and their host seeking behavior.

The direct effect of light quality on the insect visual receptors and the direct effect of temperature in insect development probably work in conjunction with the indirect effects mediated by host plants grown under a UV-deficient environment to limit growth and spatial distribution of the potato aphid. Although

further studies concerning host-insect interaction under UV-blocking covers should be conducted, our study has demonstrated that population growth and dispersal of *M. euphorbiae* is reduced under the UV-absorbing nets. As a result, the use of these covers should be recommended in an IPM strategy considering that lower pest populations and less dispersion would prevent pesticide applications and may reduce the risk of spread of aphid-borne virus diseases.

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