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RESEARCH ARTICLE

Horticultural mineral oil treatments in nurseries during aphid flights reduce *Plum pox virus* incidence under different ecological conditions

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Abstract

The application of horticultural mineral oil (HMO) treatments has been reported as a possible control strategy to reduce *Plum pox virus* (PPV) incidence in *Prunus* nurseries. The effect of Sunspray Ultrafine HMO at 1% on the natural viral spread was evaluated in experimental nursery plots of Nemaguard and Mariana GF8-1 *Prunus* rootstock blocks established under high natural inoculum pressure of the most prevalent PPV-types. Tests were conducted in experimental nursery plots in Plovdiv, Bulgaria (PPV-M and PPV-Rec), in Bistrita, Romania (PPV-D and PPV-Rec) and in Llíria, Spain (PPV-D). Horticultural mineral oil treatments were applied weekly during the vegetative period from spring to fall (treatments were interrupted in the summer). Nursery plants were analysed yearly by double-antibody sandwich enzyme-linked immunosorbent assay with 5B-IVIA/AMR monoclonal antibodies. The population dynamics of the aphids visiting plants in each experimental nursery plot was monitored by the sticky-shoot method and also by Moericke yellow water traps. At all three locations, the aphid population first peaked in the springtime. Furthermore, a variable second peak of aphid population was observed in Plovdiv and Bistrita in autumn. The treatments reduced PPV incidence in the three experimental locations and plots and in both assayed *Prunus* rootstocks grown under high PPV-inoculum pressure. A reduction from 10% to 20% of PPV-incidence between treated and control plants ($P < 0.05$) in Plovdiv and Bistrita, respectively, was observed at the end of the tests. However, HMO treatments did not prevent PPV infection altogether, probably because of the high PPV prevalence in the area near the experimental nursery blocks. The control of PPV in nursery blocks based on HMO is presented as an environmentally friendly strategy based on the physical action of the treatments.

Introduction

Plum pox virus (PPV), a member of the genus *Potyvirus*, is the causal agent of plum pox or sharka disease, the most important viral disease that affects *Prunus* (Barba *et al.*, 2011). Currently, seven PPV types or strains, that differ in their molecular and biological properties, have been reported: PPV-D (Dideron), PPV-M (Marcus), PPV-EA (El Amar), PPV-C (Cherry), PPV-W (Winona), PPV-Rec (recombinant between D and M) and PPV-T (Turkish recombinant between D and M) (Candresse & Cambra,

2006; Ulubaş Serçe *et al.*, 2009). Moreover, there are at least two more types (PPV-M Ancestral and PPV-Cherry Russian) currently under study. The most prevalent types are PPV-D, PPV-M and PPV-Rec. PPV is transmitted by graft inoculation and by different aphid species in a nonpersistent manner (Labonne *et al.*, 1995). However, the spread over long distances is mainly produced by the introduction of infected propagative plant material (Cambra *et al.*, 2006*c*).

Budsticks collected from infected trees for vegetative propagation and natural infection of rootstocks and grafted plants in nurseries constitute the main pathways for PPV introduction. Furthermore, the presence of juvenile and succulent shoots in nursery plants, ideal for aphid feeding, together with the dense block planting pattern (50 000–100 000 plants ha^{-1}) in *Prunus* nurseries, facilitate the spread of PPV among nursery blocks. The use of insecticides against viral vectors is not successful in nonpersistent virus management due to the short time between the acquisition and inoculation periods (Perring *et al.*, 1999). Moreover, the use of insecticides may have negative side effects, such as the high application cost, the potential to contribute to the spread of viral diseases by drastic reduction or disappearance of predators and parasitoids of vector species, the increase in vector activity and the occurrence of resistant vector biotypes (Fereres & Moreno, 2011). In addition, unwanted side effects, such as accumulation of toxic residues, may appear. For these reasons, alternative strategies based on physical barriers for managing PPV are essential (Vidal *et al.*, 2010).

One of the most referenced physical barriers is the use of mineral oils. Mineral oils have been used for control of a wide range of pests in different crops (Najar-Rodríguez *et al.*, 2007; Urbaneja *et al.*, 2008; Chueca *et al.*, 2009). Mineral oils act by suffocation (anoxia) (Taverner, 2002) and/or cause toxicological effects on arthropod individuals (Najar-Rodríguez *et al.*, 2008). Mineral oil treatments are being included in a large number of integrated pest and disease management programmes (Furness & Combellack, 2002). Currently, biodegradable formulations with high levels of unsulphonated residues that minimise phytotoxicity are available (Chueca *et al.*, 2009). From the lowest to the highest degree of refinement, mineral oils are classified as mineral oils, agricultural mineral oils and horticultural mineral oils (HMOs) (Kuhlmann & Jacques, 2002).

The main advantages of the use of mineral oils in plant protection are: (a) the target pests do not develop resistance due to their physical action (Chueca *et al.*, 2009); (b) beneficial arthropods, such as parasitoids, are less affected than by conventional insecticides (Urbaneja *et al.*, 2008); (c) mineral oils are not toxic to vertebrates and are quickly degraded in the environment (Najar-Rodríguez *et al.*, 2008).

Mineral oil treatments have been frequently employed for control of nonpersistent viruses (Perring *et al.*, 1999). Wang & Pirone (1996) reported that the mineral oils interfere with arthropods' ability to attach nonpersistent virus particles on the stylet and therefore reduced their transmission. Martín *et al.* (2004) also reported, for nonpersistent viruses, that oil interferes in both the acquisition and inoculation processes. Mineral oil treatments have been able to reduce the incidence of nonpersistent viruses in different horticultural crops (Simons & Zitter, 1980; Lowery *et al.*, 1990; Webb & Linda, 1993; Umesh *et al.*, 1995; Asjes & Blom-Barnhoorn, 2002). Vidal *et al.* (2010) reported that treatments based on HMO sprays were able to reduce PPV prevalence in treated blocks of Mariana GF8-1 (*Prunus cerasifera* × *Prunus munsoniana*) rootstock grown under high PPV-D inoculum pressure in Spanish Mediterranean conditions. These results encouraged more extensive experimental assays in different ecological areas, that are reported here, to assess the efficiency of HMO treatments for reducing the natural infection and spread of the most prevalent PPV types.

Materials and methods

Plant material and experimental nursery plots

Three different assays were established in different experimental nursery plots beginning in the spring of 2008. The effect of HMO on PPV incidence was evaluated in two different *Prunus* rootstocks: Nemaguard (*Prunus persica* × *Prunus davidiana*, hybrid seedling) and Mariana GF8-1, both being very susceptible to natural PPV infection (Vidal *et al.*, 2010). The first experimental nursery plot was established in Plovdiv (Bulgaria). This plot showed a warm temperate and humid climate with a hot summer (Cfa) according to the Köppen–Geiger climate classification (Kottek *et al.*, 2006). A total of 4000 plants used, 2000 with the Nemaguard rootstock and 2000 with the Mariana GF8-1 rootstock. The experimental nursery plot was established approximately 7 m from an orchard of adult European plum trees infected with PPV-M and -Rec (90% prevalence in 2008). The plot was divided into five rows separated 1.5 m from each other. Each row was formed by eight groups of 100 plants. Half of each row was sprayed with HMO, whereas the remainder was used as a control. The same number of Nemaguard and Mariana GF8-1 rootstock groups was randomly arranged in each side of each row. Therefore, half of each rootstocks were treated, while the rest remained as control.

The second experimental nursery plot was located in Bistrita (Romania). This plot showed a snowy and humid climate with a warm summer (Dfb) according to the Köppen–Geiger climate classification (Kottek *et al*., 2006). A total of 2880 plants used, 1440 with the Nemaguard rootstock and 1440 with the Mariana GF8-1 rootstock. The experimental nursery plot was established in the vicinity of two different PPV inoculum sources: a block of adult European plum trees fully infected with PPV-D and PPV-Rec and an orchard of adult European plum trees infected with both PPV-D and PPV-Rec (56% prevalence in 2008) approximately 40 m from the experimental plot. The nursery plot was divided into four blocks. Each block consisted in two rows separated 1.5 m from each other. Each row was formed by two groups of 240 plants where the two rootstock species were randomly accommodated. Groups with Nemaguard and Mariana GF8-1 plants were further subdivided lengthwise in a split-plot manner to arrange the oil treatment (oil-treated versus nontreated control).

The third experimental nursery plot was located in Llíria, Valencia (Spain). This plot showed a warm temperate climate with a dry and hot summer (Csa) according to the Köppen–Geiger climate classification (Kottek *et al*., 2006). A total of 1984 plants of Nemaguard rootstock were used. The Mariana GF8-1 rootstock was not assayed in this experimental plot because Vidal *et al*. (2010) already reported that HMO sprays reduced PPV incidence in nursery blocks of this rootstock when grown in the same ecological area. The experimental nursery plot was established approximately 5 m from an orchard of apricot trees PPV-D infected (45% prevalence in 2008). The plot was divided into four rows separated 3 m from each other. Each row was formed by eight groups of 62 plants. The HMO treatments were randomly assigned to half of the groups, while the rest remained as control.

The original rootstock plants produced in two Spanish nurseries (Viveros Orero and Agromillora Iberia), were supplied as certified PPV-free material to all the experimental plots, where the planting depth was 20 cm. All the experimental blocks were grown under standard nursery practices without any phytosanitary treatment except the HMO sprays. The rootstocks were pruned during winter and were not grafted during the experimental period.

Mineral oil treatments

The oil-spraying treatments began when the rootstocks sprouted and ended when the leaves fell. Treatments were interrupted in the summer (July and August). The treatments in Plovdiv and in Llíria ended in June 2010, while in Bistrita, they were carried out until October 2011. Sunspray® Ultrafine (HMO, 85% w/v (EC), Sun Oil Co., Philadelphia, PA, USA) used as a 1% (v/v) water emulsion was applied weekly by a spray gun (pressure of 10 bar and a spray angle of 40°) assisted by a pull-type sprayer with an agitation system.

Monitoring of PPV spread

The experimental nursery plots established in Plovdiv and Llíria were sampled and tested in spring 2009 and 2010 with one additional sampling in spring 2011 in Bistrita. Plants were individually sampled by collecting four fully expanded leaves from different parts of the canopy of each individual rootstock plant. Symptomatic leaves were collected when typical or PPV-like symptoms were observed.

Serological assays for PPV detection were performed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using the 5B-IVIA/AMR (Cambra *et al*., 1994) monoclonal antibody based kit (MagicDAS-ELISA PPV, Plant Print Diagnostics SL, Valencia, Spain) for PPV detection following the EPPO (2004) protocol.

Serological and molecular characterisation of PPV isolates

Samples from six PPV-positive plants randomly selected from the experimental nursery plots were analysed for PPV type identification according to the EPPO (2004) protocol. Three PPV isolates were collected from infected plants (positive detection) during the first and second years. Serological typing was performed by DAS-ELISA using the monoclonal antibodies 4DG5 (PPV-D specific) and AL (specific for PPV-M, PPV-Rec and PPV-T) (Plant Print Diagnostics SL). The same plant material was also analysed by molecular methods. Immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) or real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) analyses were performed with specific primers for PPV characterisation. The P1/PD, P1/PM (Olmos *et al*., 1997) and mD5/mM3 (Subr *et al*., 2004) primer pairs were used for IC-RT-PCR analyses. The PPV-MGB-F/PPV-MGB-R primers and MGB-D/MGB-M probes (Capote *et al*., 2006) were used for real-time RT-PCR analyses.

Monitoring of aphid dynamics

Adult winged-aphid species were monitored by Moericke yellow traps or by the sticky-shoot method (Avinent *et al*., 1993) in each experimental nursery plot. The time of aphid population peak was assessed.

The sticky-shoot method was used weekly in Plovdiv and Bistrita. One shoot per plant from six untreated plants per experimental plot were sprayed with glue (Souverode, Scotts and France) in the spring and autumn seasons in 2009 and 2010 in Plovdiv; and in 2008, 2009 and 2010 in Bistrita. The Moericke yellow water trap method was used in Llíria because information on the aphid visitors was already available (Capote *et al*., 2008; Vidal *et al*., 2010). Insects in the Moericke yellow traps were collected weekly from May 2008 to November 2009.

Weather data in each ecological scenario

Climate diagrams were constructed for each experimental nursery plot. The total monthly precipitation and average monthly temperature values from January 2008 to December 2010 collected from the weather stations in Plovdiv and Llíria and from January 2008 to December 2011 from the weather station in Bistrita, were used.

Statistical analysis

The effect of HMO treatments on PPV incidence in the three nursery plots was analysed per season by a generalised linear mixed model (Molenberghs & Verbeke, 2005) using rootstock and oil treatments as fixed effects and block as random effect in Bistrita. The groups of plants were included as a random effect in the statistical analysis to take into account the overdispersion phenomenon. The lme4 library of the statistical platform R (http://www.R-project.org) was used. The percentages of PPV-infected plants were assumed to follow a binomial distribution. Differences between treatment means were evaluated using mean standard errors.

Results

Characterisation of PPV isolates

The six PPV isolates collected from Plovdiv were serologically classified as PPV-M, PPV-Rec and/or PPV-T because they reacted with the 5B-IVIA/AMR and AL monoclonal antibodies but were not recognised by the type-D specific monoclonal antibody 4DG5. The six PPV isolates were molecularly amplified by real-time RT-PCR using the M-specific MGB probe but were not amplified with the D-specific MGB probe. The six isolates were classified as PPV-M because no amplification was obtained with mD5/mM3 primer pairs specific for PPV-Rec.

Four isolates out of the six PPV isolates collected from Bistrita reacted positively with the 4DG5 monoclonal antibody, and the other two isolates were recognised by the AL monoclonal antibody. Immunocapture reverse transcription-polymerase chain reaction analysis confirmed the presence of PPV-D solely in the four PPV isolates that reacted with 4DG5, while the other two PPV isolates were classified as PPV-Rec because positive amplification was only obtained with the P1/PM and mD5/mM3 primer pairs.

The six PPV isolates collected from Llíria were serologically classified as PPV-D. They reacted with the 5B-IVIA/AMR and 4DG5 monoclonal antibodies but were not recognised by the AL monoclonal antibody. The six PPV isolates were molecularly amplified by real-time RT-PCR using the D-specific MGB probes but were not amplified with the M-specific MGB probe.

Aphid dynamics and population peaks

Table 1 shows the total number of aphid individuals caught by the sticky-shoot method (Plovdiv and Bistrita) and by Moericke yellow traps (Llíria).

The total numbers of aphids caught by the sticky-shoot method in Plovdiv were 1165 in 2009 and 1777 in 2010. Two aphid population peaks were observed in 2009. The most important peak occurred in the spring from June 2 to 11. The second peak occurred during autumn from October 20 to 28. The cumulative number of aphids caught per month shows that in 2009, the highest percentage of aphids caught occurred during May and June (50.73%), followed by September and October (44.21%). In 2010, only one peak of winged aphids was detected during the spring from May 10 to 13. The highest percentage of aphid individuals caught during 2010 occurred during May and June (88.80%).

In Bistrita, the total numbers of aphids caught by the sticky-shoot method were 884 in 2008, 3081 in 2009 and 285 in 2010. Only one aphid population peak was observed in 2008 (in spring) from June 3 to 10. Similarly, only one aphid population peak was observed in spring 2009 from June 18 to 29. The highest number of aphids (83.22%) was caught in June 2009. The lowest total number of aphids caught during the three sampling periods occurred in 2010, two aphid population peaks were observed, one from June 9 to 16, and another from September 17 to 24. The highest number of aphids caught was observed in spring, during May and June (64.91% of the total captures).

In Llíria, a total of 14 366 aphids were caught by Moericke yellow traps: 4799 aphids were caught from May 2008 to December 2008 and 9567 aphids from January 2009 to October 2009. The aphid population peak was observed in spring in both sampled years. One aphid population peak was observed from May 7 to 12, 2008 and another from May 11 to 18, 2009.

Table 1 Cumulative number and percentages of aphid individuals caught by sticky shoot method and Moericke yellow traps in the experimental nursery plots established in Plovdiv (Bulgaria), Bistrita (Romania) and Llíria (Spain)

	Plovdiv[a]		Bistrita[a]			Llíria[b]	
	2009	2010	2008	2009	2010	2008	2009
January	nd[c]	nd	nd	nd	nd	nd	6 (0.06%)
February	nd	nd	nd	nd	nd	nd	22 (0.23%)
March	nd	nd	nd	nd	nd	nd	40 (0.42%)
April	nd	nd	nd	nd	nd	nd	50 0.52%)
May	155 (13.30%)	1001 (56.33%)	nd	55 (1.79%)	62 (21.75%)	4386 (91.39%)	9327 (97.49%)
June	436 (37.42%)	577 (32.47%)	561 (63.46%)	2564 (83.22%)	123 (43.16%)	90 (1.88%)	87 (0.91%)
July	12 (1.03%)	34 (1.91%)	302 (34.16%)	344 (11.17%)	28 (9.82%)	46 (0.96%)	0 (0.00%)
August	nd	nd	2 (0.23%)	0 (0.00%)	0 (0.00%)	49 (1.02%)	10 (0.10%)
September	177 (15.19%)	29 (1.63%)	14 (1.58%)	88 (2.86%)	50 (17.54%)	65 (1.35%)	17 (0.18%)
October	338 (29.01%)	109 (6.13%)	5 (0.57)	30 (0.97%)	22 (7.72%)	47 (0.98%)	8 (0.08%)
November	47 (4.03%)	27 (1.52%)	nd	nd	nd	99 (2.06%)	nd
December	nd	nd	nd	nd	nd	17 (0.35%)	nd
Total	1165 (100%)	1777 (100%)	884 (100%)	3081 (100%)	285 (100%)	4799 (100%)	9567 (100%)

[a]Total number and relative percentage of aphid individuals caught by sticky shoot method (six sticky shoots per week).
[b]Total number and relative percentage of aphid individuals caught per month by Moericke yellow trap collected weekly.
[c]Nondetermined.

Climate data

Figure 1 shows the climate diagram in the different areas where nursery experimental blocks were established from January 2008 to December 2010 in Plovdiv and Llíria, and from January 2008 to December 2011 in Bistrita. The annual average temperature and the annual average precipitation recorded from the studied period in the different nursery blocks were 12.9°C and 492 mm in Plovdiv, 10°C and 741 mm in Bistrita and 15.6°C and 481 mm in Llíria.

Effect of mineral-oil treatment on the incidence of PPV in the different experimental nursery plots

Phytotoxicity symptoms were not observed on rootstock plants in the three experimental plots. The effect of mineral-oil treatment on the spread of PPV in the different experimental nursery plots is shown in Table 2.

The percentage of PPV-infected nursery plants in treated blocks was lower than in control blocks after one year of growth in the three experimental nursery plots. Furthermore, significant differences ($P < 0.05$) between treated and control nursery blocks were found in both assayed rootstock species (Nemaguard and Mariana GF8-1) in Plovdiv after just a vegetative period and in Bistrita after two vegetative periods. The PPV incidence was so low in Llíria after one vegetative period, that no statistical analysis was performed, although the number of PPV-infected plants was higher in the control blocks (seven nursery plants) than in the treated blocks (three nursery plants). These results were confirmed later, when PPV incidence was lower in treated blocks than in control blocks, although no statistically significant differences ($P < 0.05$) in the PPV incidence were found (Table 3).

Discussion

The PPV isolates found in the three experimental nursery blocks were classified as PPV-D (Bistrita and Llíria), PPV-M (Plovdiv) and PPV-Rec (Bistrita) types, in agreement with the inoculum source present close to the plots. However, PPV-Rec was not found among the PPV-characterised rootstock plants in Plovdiv, although this isolate was present in the source of inoculum. The different benchmarks of the Köppen–Geiber classification, based on the vegetation groups present in each region together with the weather data obtained in each scenario, show that the climate conditions differed among the three experimental nursery blocks. Thus, the evaluation of the effect of HMO treatments was performed under the pressure of different PPV types and climatic conditions.

The monitoring of winged aphids and the investigation of the seasonal dynamics of their abundance are of significant importance because mainly winged morphs are responsible for the spread of nonpersistent viruses, such as PPV. The data collected on the abundance of aphid species in the experimental plots follow the basic patterns established in the population dynamics of these insects (Kindlmann *et al*., 2007). The greatest number of winged aphid individuals in the three nursery blocks was collected during the spring. This finding agrees with previous reports of the maximum peaks of aphid populations visiting adult *Prunus* orchards in southeastern

Table 2 Effect of mineral oil treatments on the spread of *Plum pox virus* (PPV) determined by DAS-ELISA 5B-IVIA/AMR in Nemaguard and Mariana GF8-1 rootstocks grown under different PPV-isolate pressure and climatic conditions

	Plovdiv (Bulgaria)			
	Spring 2009		Spring 2010	
	Nemaguard	Mariana GF8-1	Nemaguard	Mariana GF8-1
Control plants	186/924[a](0.204 ± 0.017)[b]a[c]	151/709 (0.202 ± 0.017) a	365/920 (0.416 ± 0.031) a	300/710 (0.385 ± 0.031) a
Treated plants	135/908 (0.139 ± 0.013) b	109/809 (0.137 ± 0.013) b	261/911 (0.305 ± 0.028) b	220/811 (0.278 ± 0.027) b

	Bistrita (Romania)					
	Spring 2009		Spring 2010		Spring 2011	
	Nemaguard	Mariana GF8-1	Nemaguard	Mariana GF8-1	Nemaguard	Mariana GF8-1
Control plants	31/691 (0.033 ± 0.010) a	13/693 (0.015 ± 0.005) a	229/575 (0.351 ± 0.052) a	291/686 (0.445 ± 0.055) a	299/473 (0.621 ± 0.019) a	423/654 (0.655 ± 0.016) a
Treated plants	16/709 (0.020 ± 0.006) a	9/703 (0.009 ± 0.003) a	148/658 (0.224 ± 0.040) b	233/704 (0.299 ± 0.055) b	217/522 (0.426 ± 0.019) b	323/686 (0.463 ± 0.017) b

	Lliria (Spain)	
	Spring 2009 Nemaguard	Spring 2010 Nemaguard
Control plants	7/863	224/771 (0.271 ± 0.039) a
Treated plants	3/856	191/781 (0.225 ± 0.035) a

[a]Number of PPV-infected plants/total analysed plants.

[b]Estimated mean and its standard errors (Mean ± SEM).

[c]Data in the same column followed by different letters are significantly different according to a binomial generalised linear mixed model (*P*-value < 0.05).

Table 3 Likelihood ratio tests of the generalised linear mixed model (GLMM) for treatment and rootstock effects

	Plovdiv (Bulgaria)				Bistrita (Romania)						Llíria (Spain)	
	Spring 2009		Spring 2010		Spring 2009		Spring 2010		Spring 2011		Spring 2010	
Effect	χ^{2a}	$P(>\chi^2)^b$	χ^2	$P(>\chi^2)$	χ^2	$P(>\chi^2)$	χ^2	$P(>\chi^2)$	χ^2	$P(>\chi^2)$	χ^2	$P(>\chi^2)$
Treatment	12.02	0.001	9.01	0.003	1.42	0.234	5	0.025	88.35	<2e−16	0.77	0.38
Rootstock	0.01	0.905	0.64	0.422	4.21	0.039	1.9	0.168	3.04	0.081	–	–

[a]χ^2 Likelihood ratio test.

[b]$P(>\chi^2) < 0.05$ indicate a statistically significant effect according to a binomial generalised linear mixed model.

France and Mediterranean areas of Spain (Cambra *et al*., 2006*b*; Labonne & Dallot, 2006). Moreover, a second aphid population peak, which was less important in terms of number of aphids, was observed in autumn in Plovdiv in 2009 and in Bistrita in 2010. Similar results were also reported in Hungary and Austria (Jenser *et al*., 1980; Knoll *et al*., 2004). This second peak is mainly due to the emergence of winged immigrants (gynoparae, which produce sexual females and males) and is therefore linked to the sexual reproduction and deposition of overwintering eggs. This behaviour, which is less common in Mediterranean regions, is probably more relevant in regions with cold winters, where most larvae and adults cannot survive the low temperatures and aphid individuals overwinter mainly as eggs (Knoll *et al*., 2004). Literature shows that when a higher aphid population peak is observed in the spring, the peak in autumn is usually less pronounced (Kindlmann *et al*., 2007). In this regard, fewer aphids were collected in spring 2009 than in the spring 2010 in Plovdiv, while the autumn population peak in 2009 was lower than in 2010. The data from Bistrita suggest this same pattern.

The population dynamics of aphid species can vary depending on the year and other factors such as weather conditions, natural enemies, abundance in the previous years or human action (Dixon, 1985; Sequeira & Dixon, 1997; Leslie *et al*., 2009). This fact could explain the variation in the number of aphid individuals caught in the different experimental nursery blocks in different years.

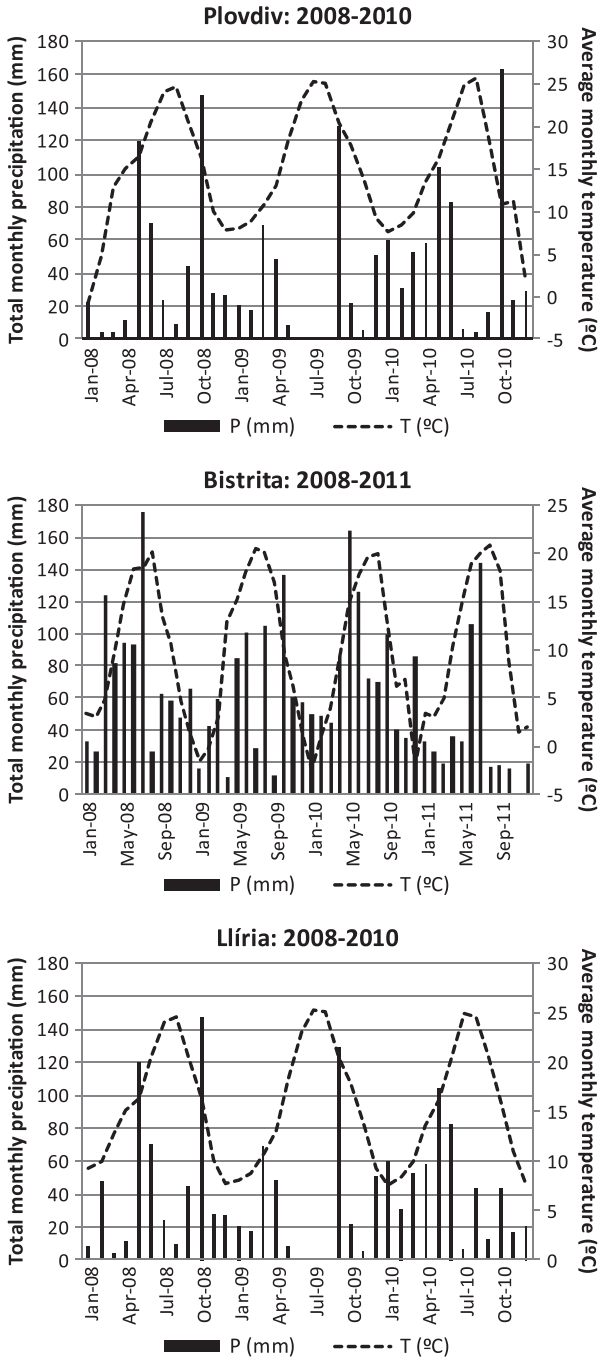

Figure 1 Climate diagram: Plovdiv, Bistrita and Llíria.

The HMO treatments significantly reduced PPV infection in both assayed rootstock species (Nemaguard and Mariana GF8-1) in two of the three experimental nursery plots. PPV incidence was always lower in treated blocks than in control blocks. These results confirm those previously reported by Vidal *et al*. (2010), who observed that the HMO treatments reduced PPV incidence in treated blocks under continental moderate climatic conditions with the PPV-D type as the inoculum source in Nemaguard and Mariana GF8-1 rootstocks, obtaining significant differences in the Mariana GF8-1 blocks after two vegetative periods. Furthermore, HMO treatments reduced PPV incidence in experimental nursery plots located in areas where different PPV types prevail. This fact could be due to the physical action of HMO. These results indicate that HMO treatments can be used for the control and management of PPV spread independent from the PPV type or isolates present in a particular area.

Horticultural mineral oil treatments were able to reduce PPV infection in the treated blocks, but they were not able to avoid the PPV infection. Two factors could explain this fact: (a) the high inoculum pressure present in the experimental nursery plots and/or (b) the close proximity of the control (nontreated) and sprayed blocks (Simons & Zitter, 1980; Webb & Linda, 1993). Simons & Zitter (1980) have reported that under the ecological conditions present in Florida (USA), mineral oil is ineffective against nonpersistent viruses in horticultural crops when the level of infection is between 10% and 20%. Therefore, the high inoculum pressure present in the experimental nursery plots might explain the modest reduction (between treated and control plants) in PPV prevalence. In addition, the very close proximity of the control and sprayed plant plots could explain the presence of PPV-infected plants in treated blocks because the oil-treated plants were constantly exposed to aphids that could have acquired the virus (PPV-viruliferous aphids) from adjacent untreated plants (Webb & Linda, 1993). This could lead to underestimating the ability of HMO treatments to reduce PPV spread in nursery blocks. In this regard, an experimental design with more frequent inspection/testing and removal of infectious plants could provide a more accurate estimation of the effect of HMOs on PPV contamination from external sources. Horticultural mineral oils may reduce virus spread by acting both against acquisition and inoculation processes, but in our experimental nursery blocks, only inoculation was affected by HMOs in a realistic manner (because acquisition can happen on untreated plants). Plant material must therefore, be established the furthest away from any PPV-infected *Prunus* host as possible to avoid potential contamination. The use of preventive HMO treatments should contribute to preventing occasional PPV infections that could happen in PPV-free areas or in areas where sporadic PPV outbreaks occur. Furthermore, in areas with an endemic presence of PPV, the HMO treatments presented herein have shown to reduce PPV spread and incidence and could be used combined with other measures such as buffer zones (PPV-free areas) to

control PPV infection in nursery blocks (Boscia *et al.*, 2012).

According to our results, the HMO treatments must be applied at least weekly in the springtime under European conditions, although in colder regions, it might be necessary to apply HMO treatments in the autumn as well. Moreover, aphid population dynamics can vary in different years. For that reason, monitoring methods, such as Moericke yellow traps or sticky shoots, must be constantly applied to assess the flights of the aphid species present in the crop before applying HMO treatments. The early detection of PPV-viruliferous aphids landing on a nursery block (Cambra *et al.*, 2006*a*) could be used as a warning signal and as an indication for starting the HMO treatments. The absence of PPV-viruliferous aphids indicates that the treatments should be ended. In areas with a low risk of infection, the treatments might be limited to the aphid population peaks.

Moreover, the efficiency of treatments depends on the way in which they are distributed on the plant canopy, which is in turn directly related to the application equipment and its setup (Chueca *et al.*, 2009). It is therefore crucial to implement the available technologies for mineral oil application in stone fruit nurseries to improve the efficiency of the treatment (Vidal *et al.*, 2010).

In summary, HMO treatments can reduce PPV incidence by 10–20% under different ecological scenarios. These results indicate that the application of HMO treatments could be recommended for the control of PPV in nursery blocks, together with other complementary actions, such as the location of nurseries in PPV-free areas, the separation of nursery facilities from PPV-infected hosts, the use of rootstocks that are less susceptible to natural PPV infection, and/or the cultivation of nursery mother plants under insect-proof protection or facilities. In addition, the current reduction of authorised active pesticide substances (Council Directive 91/414 ECC) will probably lead to HMO treatments being one of the few phytosanitary authorised products in the near future. Consequently, HMO treatments can be incorporated into any integrated control strategy of PPV in nursery blocks. The use of HMO treatments could equally reduce PPV spread in newly established orchards during the first years after planting.

Furthermore, a new type of oils is coming on the market (plant oils), which could offer new opportunities for the control of nonpersistently transmitted virus.

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