horticulturae

MDPI

Article

The Influence of Crop Habitat and Control Strategies on Pepper Viruses in Andalusia (Spain)

Almudena Simón, Carmen García, Fernando Pascual, Leticia Ruiz and Dirk Janssen *

Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Centro La Mojonera, Camino de San Nicolas 1, La Mojonera 04745, Spain; almudenasimonmartinez@gmail.com (A.S.); mariac.garcia.g@juntadeandalucia.es (C.G.); fervirologia@hotmail.com (F.P.); leticia@thetemplatestore.com (L.R.)

* Correspondence: dirk.janssen@juntadeandalucia.es; Tel.: +34-67-059-3012

Academic Editors: Elazar Fallik and Douglas D. Archbold

Received: 4 July 2016; Accepted: 7 October 2016; Published: 14 October 2016

Abstract: Andalusia, southern Spain, is a major horticultural production region within the Mediterranean, where over 10,000 ha are dedicated to the production of pepper (*Capsicum annuum* L.). Approximately two-thirds of the area dedicated to this crop is in a greenhouse and the remaining one-third is comprised of open field crops. Using pepper as a model, we identified and compared the major diseases caused by viruses in the different geographic regions and agronomic systems within the region. Symptomatic samples were collected during 2009 and analyzed by ELISA and RT-PCR for the presence of *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV), *Tomato mosaic virus* (ToMV), *Pepper mild mottle virus* (PMMoV), *Potato virus Y* (PVY), *Tobacco mild green mosaic virus* (TMGMV), *Tomato chlorosis virus* (ToCV) and *Parietaria mottle virus* (PMoV). Contingency table analysis showed a significant relationship between the presence of major diseases caused by viruses in pepper crops and the different agrosystems in terms of location (inland versus coastal), disease control management (chemical versus integrated), cropping system (open field versus greenhouse), and virus-resistant versus susceptible cultivars. Pepper crops in plastic-covered greenhouses were predominantly associated with arthropod-transmitted virus diseases, such as TSWV. CMV was predominant in provinces located inland, and PMoV was found independent of the agrosystem, disease control methods, or geographic location.

Keywords: *Capsicum annuum*; *cucumber mosaic virus*; *parietaria mottle virus*; *potato virus Y*; tobamovirus; *tomato spotted wilt virus*; greenhouse

1. Introduction

Increasing numbers of virus species cause diseases in horticulture crops worldwide. These crops are grown in different geographic locations, each with their own environmental and agro-technical features. Consequently, there exists an infinite range of agrosystems developed to reduce the effect of pests and diseases and to improve agronomic productivity. These agrosystems could be considered ecological communities in which plant pathogens have their own dynamics, defined for example by host suitability, climate, and vectors [1]. In the case of plant viruses that are transmitted by vectors, their management can involve conventional (chemical) control and/or integrated (including biological) control [2]. The use of virus-resistant cultivars constitutes an important means to control virus diseases [3]. Greenhouses and other protected horticultural environments allowing year-round production offer some physical protection against arthropods and diseases. An element determining the success of control can be the existence of arthropod pest and disease reservoirs surrounding the greenhouses, associated with wild vegetation, or with open-field cultivated crops [4]. This also holds for plant viruses, and examples exist where the incidence in outdoor crops during seasonal periods is

believed to affect that in nearby greenhouses during off-season production such as in Mediterranean horticultural production where summer open-field and greenhouse winter crops often overlap [5].

Pepper (*Capsicum annuum* L.) production in Spain ranks fifth on a global scale. The Region of Andalusia (southern Spain) has over 10,900 ha dedicated to pepper, and most is cultivated in greenhouses (about 7500 ha) which include various "parral" type and multi-tunnel houses located along the coast of the province of Almeria. The remaining 3400 ha are produced outdoors in the remaining seven provinces [6]. Greenhouse-grown cultivars are planted mostly for fresh consumption and open-field crops are for the food processing industry. Modern horticulture is performed in highly intensive farming systems, but often production suffers heavy losses from arthropod pests and diseases [7], and in the case of pepper, these losses are due to disease caused by plant viruses. Some of these viruses are contact and seed-transmitted, including *Tomato mosaic virus* (ToMV, [8]), *Pepper mild mottle virus* (PMMoV, [9]) and *Tobacco mild green mosaic virus* (TMGMV, [10]), and some are aphid transmitted, including *Cucumber mosaic virus* (CMV, [11]) and *Potato virus Y* (PVY, [11]), and have been present in the region and crop species for many decades. Others are considered emerging viruses and include the western flower thrips *Frankliniella occidentalis*-transmitted *Tomato spotted wilt tospovirus* (TSWV, [12]), *Bemisia tabaci*-transmitted *Tomato chlorosis crinivirus* (ToCV, [13]) and the pollen-transmitted *Parietaria mottle ilarvirus* (PMoV, [14,15]).

Here, relationships between the presence of major diseases caused by viruses in pepper crops and the different agrosystems in terms of location (inland versus coastal), disease control management (chemical versus integrated), cropping system (open field versus greenhouse), and virus-resistant versus susceptible cultivars, are examined.

2. Materials and Methods

2.1. Sample Collections

During 2010, samples were collected from crops located in 71 towns and villages in Andalusia, distributed among 18 different pepper producing regions within the provinces of Almeria, Granada, Malaga, Cadiz, Huelva, Seville, Cordoba and Jaen (Figure 1). The numbers of samples collected per ha of crop surface corresponded to at least 2.5% of the relative crop surface dedicated to pepper in each of the eight provinces, according to available statistical information [6] (Table 1). The sampled crops were characterized and labelled according to the administrative location (province), geographic origin as either coastal (H1, C1, M1, G1, A1 and A2, in Figure 1) or inland (S1, M2, M3, Co1, Co2, J1, J2, G2, G3, G4, A3 and A4), the type of farm (crops grown in a greenhouse or in the open field), the plant health control strategy applied (arthropod pest control based on chemical treatments, or on integrated pest control which includes biocontrols, as determined after visual inspection of the crops and interviewing the farmers), and the crop cycles (crops planted in autumn or in spring).

Table 1. Area (ha) of pepper crops under cultivation in Andalusia during 2009 [6] and the numbers of samples collected for the present study.

Province	Open Field	Protected	Total	Samples
Almeria	73	7432	7505	186
Cadiz	902	–	902	27
Cordoba	593	–	593	15
Granada	264	306	570	22
Huelva	155	4	159	4
Jaen	264	–	264	8
Malaga	156	590	745	24
Seville	183	25	208	11
Andalusia	2598	8357	10,946	297

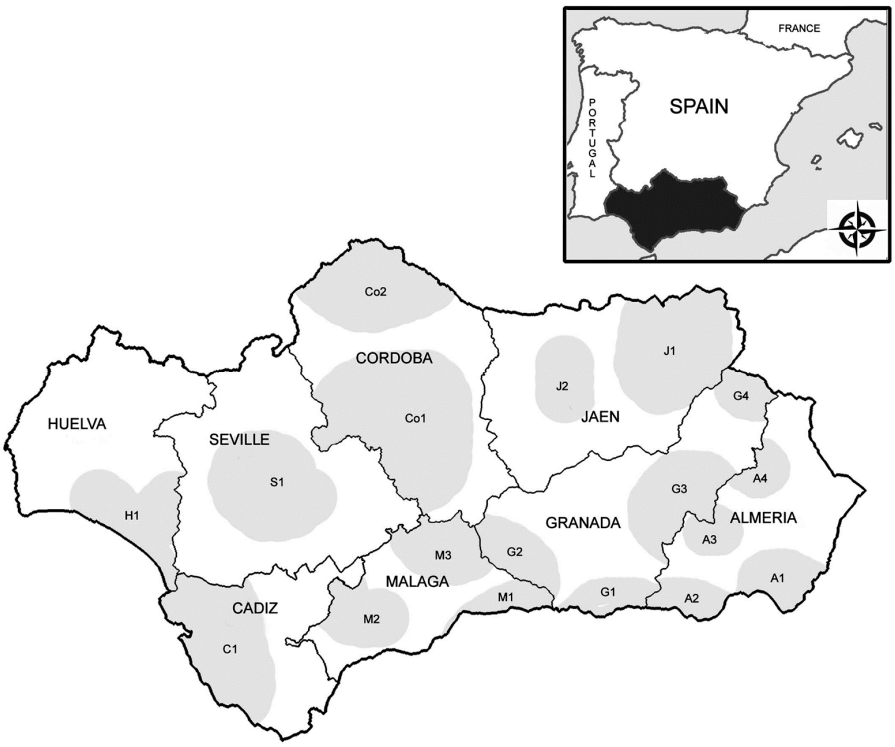

Figure 1. Map of Andalusia with pepper producing areas where virus surveys were undertaken during the year 2010. The main towns located within the sampling zones were (A1) Nijar, Almeria, (A2) El Ejido, Roquetas de Mar, Adra, (A3) Laujar, Sta. Cruz (A4) Tijola, (G1) Albuñol, Motril, (G2) Alhama de Granada, Zafarraya, (G3) Guadix, Darro, Baza, (G4) Cullar, Puente de Don Fabrique, (M1) Torrox, Velez Malaga, (M2) Coin Alhaurin de la Torre, Ronda, (M3) Antequera, Archidona, (C1) San Lucar de Barrameda, Chipiona, Vejer de la Frontera, (J1) Cazorla, Úbeda, (J2) Jaen, Bailen, (Co1) Córdoba, Puerto Genil, Santaella, (Co2) El Viso, Hinojosa del Duque, (S1) Burguillos, Carmona, La Rinconada, and (H1) Hinojosa, Moguer, Almonte.

Sampled pepper plants belonged to one of a wide range of cultivar types such as Lamuyo, Bell Pepper, Kappya, Italian Sweet Pepper, Chili pepper, and snack-sized peppers, and were from either local or commercial cultivars, including varieties from the following seed companies: De Ruiter Seeds, Enza Zaden España, Nunhems Spain S.A, Rijk Zwaan Iberica S.A., Semillas Fitó, Syngenta Seeds, Tozer Seeds Iberica, Western Seed 2000 SL, Zeraim Ibérica S.A., and Zeta Seeds. Samples consisted of fruit and leaves from plants displaying symptoms suggesting virus infection, including leaf mosaic, ring and leaf spotting, leaf deformation, leaf puckering, and/or plant stunting. All samples were kept at 4 °C and were analyzed 2–5 days after collection.

2.2. Virus Detection

All samples were analyzed for the presence of TSWV, CMV, ToMV, PMMoV, TMGMV and PVY using ELISA and specific antisera from Loewe Biochemica GmbH (Sauerlach, Germany) following the manufacturer's instructions. PMoV and ToCV were detected using RT-PCR: total RNA was purified from 0.2 g leaf tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. RT-PCR reactions were developed with the Titan One Tube RT-PCR system (Roche) employing total RNA preparations from pepper and following manufacturer's instructions. PMoV was amplified using the following primers as described (5′-GATGTTGCCGCCGACGATTCTA-3′ and 5′-TTTTCCCACAACCCGAACAC-3′), designed after Genbank Sequence Accession No. AY496068. These primers produce an amplicon of the expected size (475 bp) [14]. ToCV was detected with the primers 5′-TTTGTTCCTCTTTGGGTTTC-3′ and 5′-ATGGGTTTTCTGATGATAAT-3′, designed to

amplify a 708-bp fragment from the ToCV genome [9]. The PCR conditions to generate PMoV and ToCV amplicons were 95 °C for 3 min, 35 cycles of denaturation for 20 s at 95 °C, annealing for 30 s at 55 °C and extension for 40 s at 72 °C followed by one final extension cycle for 8 min at 72 °C.

2.3. Statistics

From the number of samples with viruses, contingency tables were constructed, and the Fisher's exact test for equality of distributions was used to investigate whether the resulting proportions could be explained based on different factors, such as geographic origin, climate, crops producing, as well as arthropod pest and disease control systems.

3. Results

Table 1 summarizes the numbers of pepper samples collected and their geographic location. All of the samples were negative for ToCV. TSWV was the virus found most frequently (71% of samples), followed by CMV and PMoV with similar frequencies of 17% and 16.5%, respectively. ToMV, PMMoV and PVY were less frequently detected: 4.71% of samples contained one of the tobamoviruses (ToMV, PMMoV or TMGMV), and only 1.35% of samples had PVY. All of these virus species are commonly found in Andalusia although not all viruses were detected in all provinces.

From the collected samples, 268 (90.23%) proved to be infected by at least one of the virus species analyzed. In samples collected from most of the Andalusian provinces, at least two viruses were detected, either as simple or as double infections (Table 2). In the coastal provinces of Almeria, Granada, Malaga and Cadiz, TSWV was the most prevalent virus (88.71%, 63.64%, 54.17% and 92.59% respectively). Whereas the percentage of CMV in Almeria was only 2.69%, it was 22.73% in Granada, and 54.17% in Malaga. In contrast, the percentage of PMoV was similar in Almeria (23.66%) and Granada (27.27%). That of ToMV was only 4.17% in Malaga. The situation is different for the inland provinces of Seville, Cordoba and Jaen where CMV was the prevalent virus, detected in 81.82% of samples from Seville, in 73.33% of samples from Cordoba and in 63.64% of the samples in the province of Jaen. Interestingly, PVY and PMMoV were detected in 36.40% and 27.27%, respectively, of samples from Seville (Table 2).

Table 2. Percentages of collected samples with single and mixed infections of viruses detected from pepper crops of the eight provinces of Andalusia.

	Province							
	Almeria	**Granada**	**Malaga**	**Cadiz**	**Huelva**	**Seville**	**Cordoba**	**Jaen**
Viruses	%							
One Infection								
TSWV	68.98	54.54	41.67	92.59	0	0	0	0
CMV	1.6	0	41.67	0	0	9.09	73.33	62.5
PMoV	4.81	0	0	0	0	0	0	0
Two Infections								
TSWV + CMV	0.53	0	8.33	0	0	0	0	12.5
TSWV + PMoV	17.65	4.54	0	0	0	0	0	0
CMV + PMoV	0	18.18	0	0	0	0	0	0
CMV + TMGMV	0	0	0	0	0	36.36	0	12.5
Three Infections								
TSWV + CMV + ToMV	0	0	4.17	0	0	0	0	0
TSWV + PMoV + CMV	0.53	4.54	0	0	0	0	0	0
TSWV + PMoV + TMGMV	0.53	0	0	0	0	0	0	0
CMV + PVY + TMGMV	0	0	0	0	0	9.09	0	0
Four Infections								
CMV + PVY + PMMoV + TMGMV	0	0	0	0	0	27.27	0	0
No Virus	5.35	18.18	7.17	7.41	100	18.18	26.67	12.5

TSWV, *Tomato spotted wilt virus*; ToMV, *Tomato mosaic virus*; PMMoV, *Pepper mild mottle virus*; TMGMV, *Tobacco mild green mosaic virus*; PMoV, *Parietaria mottle virus*; CMV, *Cucumber mosaic virus*; PVY, *Potato virus Y*.

Of all samples 71.38% were from cultivars that were resistant to one or several of the viruses studied, whereas 22.90% were from cultivars without any virus resistance (Table 3). Virus resistant cultivars were almost completely restricted to the greenhouses located along the coastal provinces (Almeria, Granada, Malaga and Cadiz). Although cultivars resistant to CMV were commercially available, none of the collected samples had CMV-resistance genes. At present, there are no commercial cultivars that are resistant to PMoV. As for all of the inland provinces (Seville, Cordoba and Jaen), the collected samples were from plants that had no resistance gene. In regions where PVY, ToMV and PMMoV were found, no cultivars resistant to tobamovirus were commonly used (Table 3).

Table 3. Percentages of collected samples with and without resistance to the viruses studied in the pepper crops from the eight provinces of Andalusia.

	Province							
	Almeria	**Granada**	**Malaga**	**Cadiz**	**Huelva**	**Seville**	**Cordoba**	**Jaen**
Virus-Resistance	%							
TSWV	4.84	9.09	0	0	0	0	0	0
PVY	4.84	0	8.33	0	0	0	0	0
ToMV	10.75	36.36	0	0	100	0	0	0
ToMV + PMMoV	17.74	4.54	0	74.07	0	0	0	0
TSWV + ToMV	13.44	0	0	0	0	0	0	0
TSWV + ToMV + PVY	5.38	0	0	0	0	0	0	0
TSWV + ToMV + PMMoV	22.58	9.09	0	0	0	0	0	0
ToMV + PMMoV + PVY	9.14	0	0	0	0	0	0	0
TSWV + ToMV + PMMoV + PVY	4.30	0	0	0	0	0	0	0
Local cultivars, without resistance	3.76	22.73	91.67	0	0	100	100	100
Unknown cultivars	3.22	18.18	0	25.93	0	0	0	0

TSWV, *Tomato spotted wilt virus*; ToMV, *Tomato mosaic virus*; PMMoV, *Pepper mild mottle virus*; TMGMV, *Tobacco mild green mosaic virus*; PMoV, *Parietaria mottle virus*; CMV, *Cucumber mosaic virus*; PVY, *Potato virus Y*.

All of the descriptive data linked to the pepper samples, geographic origin, climate, other crops being produced, as well as arthropod pest and disease control systems, were added to contingency tables and subjected to the Fisher's exact test for equality of distributions in order to investigate whether the resulting proportions could be explained based on these diverse factors. The results (Table 4) show that most viruses were specifically detected according to location, cropping system, disease control strategy, and crop cycles ($\alpha < 0.05$). Only PMoV was independent of whether the crops were coastal or inland located, open field or greenhouse-grown, and autumn-winter or spring-summer crop cycles. Tobamoviruses were independent only for the crop cycle period.

Table 4. Table of the relationships [a] and statistical significance among viruses detected during a survey of pepper crops and the different geographic locations, farm types, pest and disease control methods, and crop cycles.

	CMV + PVY	**ToMV + PMMoV + TMGMV**	**PMoV**	**TSWV**
Number of samples	54	14	49	211
Location: coastal versus inland	<0.01 **,[b]	0.03 **	1.70 ns	59.71 **
Farm type: greenhouse versus open field	<0.01 **	0.02 **	2.35 ns	263.90 **
Pest/Disease control: chemical versus integrated	38.60 **	0.29 *	39.00 **	0.04 **
Crop cycle: spring versus autumn	81.34 **	0.59 ns	0.59 ns	0.02 **

[a] Expressed as relative proportions; [b] ns = not significant, $\alpha \geq 0.05$; * $\alpha < 0.05$; ** $\alpha < 0.0001$ (Fisher's exact test for equality of distributions).

4. Discussion

In Andalusia, horticulture crops are produced in two cropping agrosystems [16,17]. One type is located in the inland regions with a sub-continental climate, having cold winters and hot summers, and in which crops are grown outdoors using cultivars without resistance to pathogens. In some cases, seeds are extracted and retained for the next crop. While obtained from selected plants and apparently

healthy fruit, they lack preventive disease treatments. In this type of farm, the crop cycle takes place during the warmer months of the year. Fruit yields suffer considerable quality losses from viruses and other diseases, and they are largely sold locally. The second system is located along the coastal areas of the provinces of Almería, Granada, Malaga and Cadiz (Figure 1). The latter province hosted over 1000 ha of cut flower production since the 1980's, but that has gradually been replaced by vegetable crops over the last ten years. In Malaga, subtropical cultures predominate. All of these areas have mild winter climates that permit greenhouse production during autumn-winter and even during spring. Arthropod pest control is based on integrated management and includes cultural practices, mechanical insect exclusions (including insect screens on lateral and roof windows), and biological control [18]. On these farms, high quality commercial seeds are used that are F1 hybrids that have been chemically treated and contain resistance genes against diseases [19]. The yields obtained are generally high and the produce is sold on the national and European market [20].

In contrast to other parts of the world, the most prevalent viruses in the Mediterranean over the past 20 years have been mostly restricted to two tobamoviruses, several aphid-transmitted potyviruses, CMV, and thrips-transmitted TSWV [21]. The relatively constant plant health situation means that pepper could also serve as a model to compare pathological situations within and between production areas. The main viruses in pepper from France are CMV, PVY, TMV, PMMoV and TSWV [22]. In our study, CMV was present in all provinces except Cadiz (Table 2). This virus is non-persistently transmitted by aphids, mostly of the species *Aphis gosspii* and *Myzus persicae*. CMV has a very broad host range, including member species of the *Solanaceae, Cucurbitacae, Leguminosae* and many spontaneous plant species [23]. This wide host range leads to extensive virus reservoirs close to horticultural crops. In Almeria, most greenhouses have insect screens of 10 $\times$ 20 threads/cm^2 that exclude aphids that reside outside the greenhouse. However, in Malaga and Granada, most of the farms produce open field crops where CMV reaches higher incidences.

We found that, within Andalusia, open field pepper crops suffered mostly from seed-transmitted viruses and those that are aphid-transmitted such as CMV (Tables 2 and 4). Similar results were obtained for field-grown pepper in Turkey with PVY and TMV in double infections [24], or with PVY and ToMV in Ethiopia [25], with PVY and CMV in Serbia [26], and in tomatoes in Valencia (Spain) where PVY and ToMV were the predominant viruses [5]. The inland climatic conditions of the Andalusian regions where these field pepper crops were located did not favor the presence of *F. occidentalis* thrips and the transmission of TSWV. During the summer, high temperatures (>40 °C) and low humidity (~20%), which can produce a drastic reduction in the populations of thrips [27,28], are common. On the other hand, crops produced in greenhouses without these climate extremes had high incidences of diseases caused by viruses such as TSWV. These crops were physically protected against invading aphids using insect screens, and therefore viruses such as CMV were rarely in evidence.

Populations of viruses carried in seed or transmitted by vectors to field tomato crops can determine levels of infection of greenhouse-grown tomato during a consecutive season [5]. In particular, arthropods cross boundaries between different habitats [29], and this could affect the transmission of viruses as they may be vectors, and influence the response of their predators and parasitoids. For pepper crops we would expect pathogen spillover might take place, defined as when the epidemics in a host population are driven not by transmission within that population but by transmission from a reservoir population [30]. However, from our work, most virus infections appeared specific to location, cropping system, disease control strategy and crop cycles (Table 4). Probably, the recent large-scale application of integrated pest management in pepper greenhouses explains the significant association between the presence of TSWV and the type of disease management [18]. The use of cultivar resistance against tobamoviruses has proven effective as well. These viruses are rarely found in greenhouses from Almeria where resistance has been used since 2000 [31] (Table 2). The presence of tobamoviruses was related to the location and type of farms as well as the type of plant health control used, e.g., the presence or absence of cultivar resistance and the use of untreated saved seed.

Of all the viruses surveyed, PMoV was the only virus detected in pepper crops irrespective of the geographic and agronomic parameters. PMoV was found inland and at the coast of Andalusia, both in open field and in greenhouse crops, and in summer and winter crops (Table 4). To date, no pepper cultivars resistant to PMoV are available. PMoV has emerged as a pathogen of Solanaceous crops in the Mediterranean countries of Spain, France, Italy and Greece [14,32–34]. Recent studies suggest that pollen transmitted from surrounding *Parietaria officinalis* plants could be a major source of infection in Solanaceous crops, and several insect species of the orders Hemiptera and Thysanoptera were candidates as vectors. These include biological control agents such mirids that are predators of arthropod pests [15]. These features within the context of integrated pest management in greenhouses could pose one of the greatest challenges for successful control of PMoV among all of the viruses of pepper in Andalusia.

5. Conclusions

The present study compared virus species found in pepper crops that were grown in different locations, in open air fields and in greenhouses, with chemical or with integrated disease control, and as summer or as winter crops. During this survey in southern Spain, significant differences were evident as aphid-transmitted CMV was mostly found in open field crops. Only few cases of infection with tobamoviruses ToMV and PMMoV were registered, probably since most farms use tobamovirus-resistant cultivars. Trips-transmitted TSWV was found in greenhouses located near the coast, where no extreme temperatures and percentages of humidity occur that can harm the vector. In contrast, PMoV, which is transmitted by pollen and to which there are no resistant cultivars, was detected in pepper crops irrespective of the geographic and agronomic parameters, and therefore constitutes an emerging virus that could pose a serious threat to pepper crops in the Mediterranean.

Acknowledgments: This study was financed by project INIA-FEDER: RTA09-00016-00-00. The research contract of L.R. is 80% supported by the Andalusian Operational Programme, European Social Fund (2007–2013). We thank Manuel Arriaza (IFAPA, Centro Alameda del Obispo) for help on the statistical analyses of data, and Vince Reid for advice during the preparation of the manuscript.

Author Contributions: D.J. and C.G. conceived and designed the experiments; A.S. and F.P. performed the experiments; A.S. and D.J. analyzed the data; L.R. contributed analysis tools; D.J. and A.S. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Malmstrom, C.M.; Melcher, U.; Bosque-Pérez, N.A. The expanding field of plant virus ecology: Historical foundations, knowledge gaps, and research directions. *Virus Res.* **2011**, *159*, 84–94. [CrossRef] [PubMed]
2. Reitz, S.R.; Yearby, E.L.; Funderburk, J.; Stavisky, J.; Olson, S.M.; Momol, M.T. Integrated management tactics for *Frankliniella thrips* (Thysanoptera: Thripidae) in field-grown pepper. *J. Econ. Entomol.* **2003**, *96*, 1201–1214. [CrossRef] [PubMed]
3. Pitrat, M. Vegetable crops in the Mediterranean Basin with an overview of virus resistance. *Adv. Virus Res.* **2012**, *84*, 1–29. [PubMed]
4. Berlinger, M.J.; Jarvis, W.R.; Jewett, T.J.; Lebiush-Mordechi, S. Managing the greenhouse, crop and crop environment. In *Integrated Pest and Disease Management in Greenhouse Crops*; Albajes, R., Lodovica Gullino, M., van Lenteren, J.C., Eds.; Kluwer Academic Publishers: New York, NY, USA, 2002; pp. 97–123.
5. Soler, S.; Prohens, J.; López, C.; Aramburu, J.; Galipienso, L.; Nuez, F. Viruses infecting tomato in Valencia, Spain: Occurrence, distribution and effect of seed origin. *J. Phytopathol.* **2010**, *158*, 797–805. [CrossRef]
6. Ministerio de Medio Ambiente y Medio Rural y Marino. *Análisis Provincial de Superficie, Rendimiento y Producción*; Ministerio de Medio Ambiente y Medio Rural y Marino: Madrid, Spain, 2009. (In Spanish)
7. Cuadrado, I.M. Situación de las virosis en la horticultura almeriense. *Vida Rural* **1999**, *97*, 38–39. (In Spanish)
8. Alonso, E.; Garcia Luque, I.; Avila Rincón, M.J.; Wicke, B.; Serra, M.T.; Diaz Ruiz, J.R. A tobamovirus causing heavy losses in protected pepper crops in Spain. *J. Phytopathol.* **1989**, *125*, 67–76. [CrossRef]

9. Velasco, L.; Simón, B.; Janssen, D.; Cenis, J.L. Incidences and progression of tomato chlorosis virus disease and tomato yellow leaf curl virus disease in tomato under different greenhouse covers in southeast Spain. *Ann. Appl. Biol.* **2008**, *153*, 335–344. [CrossRef]
10. Luis Arteaga, M.; Saez, E.; Berdiales, B.; Rodriguez Cerezo, E. Detección del virus del moteado verde atenuado del tabaco (TMGMV) en cultivos de berenjena y pimiento en Almería. In Proceedings of the Ninth Congress, Salamanca, Spain, 19–23 October 1998; Sociedad Española de Fitopatologia: Madrid, Spain, 1998; p. 69. (In Spanish)
11. Avilla, C.; Collar, J.L.; Duque, M.; Fereres, A. Yield of bell pepper (*Capsicum annuum*) inoculated with CMV and/or PVY at different time intervals. *Z. Pflanzenkrankh. Pflanzenschutz* **1997**, *104*, 1–8.
12. Jorda, C.; Nuez, F.; Lacasa, A. Situación actual de la virosis del bronceado en los cultivos españoles. *Phytoma Esp.* **1995**, *66*, 20–23. (In Spanish)
13. Fortes, I.M.; Moriones, E.; Navas Castillo, J. Tomato chlorosis virus in pepper: Prevalence in commercial crops in southeastern Spain and symptomatology under experimental conditions. *Plant Pathol.* **2012**, *61*, 994–1001. [CrossRef]
14. Janssen, D.; Sáez, E.; Segundo, E.; Martín, G.; Gil, F.; Cuadrado, I.M. *Capsicum annuum*—A new host of Parietaria mottle vírus in Spain. *Plant Pathol.* **2005**. [CrossRef]
15. Aramburu, J.; Galipienso, L.; Aparicio, F.; Soler, S.; López, C. Mode of transmission of *Parietaria mottle virus*. *J. Plant Pathol.* **2010**, *92*, 679–684.
16. Aznar-Sánchez, J.A.; Galdeano-Gómez, E.; Pérez-Mesa, J.C. Intensive horticulture in Almería (Spain): A counterpoint to current European rural policy strategies. *J. Agrar. Chang.* **2011**, *11*, 241–261. [CrossRef]
17. Leyva, R.; Constán-Aguilar, C.; Blasco, B.; Sánchez-Rodríguez, E.; Romero, L.; Soriano, T.; Ruíz, J.M. Effects of climatic control on tomato yield and nutritional quality in Mediterranean screenhouse. *J. Sci. Food Agric.* **2014**, *94*, 63–70. [CrossRef] [PubMed]
18. Glass, R. Biological control in the greenhouses of Almeria and challenges for a sustainable intensive production. *Outlooks Pest Manag.* **2012**, *23*, 276–279. [CrossRef]
19. Córdoba-Sellés, C.; Cebrián, M.C.; Sánchez-Navarro, J.A.; Espino, A.; Martín, R.; Jordá, C. First report of Tomato torrado virus in tomato in the Canary Islands, Spain. *Plant Dis.* **2007**. [CrossRef]
20. Galdeano-Gómez, E.; Aznar-Sánchez, J.A.; Pérez-Mesa, J.C. Sustainability dimensions related to agricultural-based development: The experience of 50 years of intensive farming in Almeria (Spain). *Int. J. Agric. Sustain.* **2013**, *11*, 125–143. [CrossRef]
21. Moury, B.; Verdin, E. Viruses of pepper crops in the Mediterranean basin: A remarkable stasis. *Adv. Virus Res.* **2012**, *84*, 127–162. [PubMed]
22. Marchoux, G.; Ginoux, G.; Morris, C.; Nicot, P. Pepper: The breakthrough of viruses. *PHM Rev. Hortic.* **2000**, *410*, 17–20.
23. Zitter, T.A.; Murphy, J.F. Cucumber mosaic. *Plant Health Instr.* **2009**. [CrossRef]
24. Arli-Sokmen, M.; Mennan, H.; Sevik, M.A.; Ecevit, O. Occurrence of viruses in field-grown pepper crops and some of their reservoir weed hosts in Samsun, Turkey. *Phytoparasitica* **2005**, *33*, 347–358. [CrossRef]
25. Hiskias, Y.; Lesemann, D.-E.; Vetten, H.J. Occurrence, distribution and relative importance of viruses infecting hot pepper and tomato in the major growing areas of Ethiopia. *J. Phytopathol.* **1999**, *147*, 5–11. [CrossRef]
26. Petrovic, D.; Bulajic, A.; Stankovic, I.; Ignjatov, M.; Vujakovic, M.; Krstic, B. Presence and distribution of pepper viruses in Serbia. *Ratar. i Povrt.* **2010**, *47*, 567–576.
27. Contreras, J.; Pedro, A.; Sanchez, J.A.; Lacasa, A. The influence of extreme temperatures on the development of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). (Influencia de las temperaturas extremas en el desarrollo de *Frankliniella occidentalis* (Pergande) (Thysanoptera: *Thripidae*)). *Bol. Sanid. Veg. Plagas* **1998**, *24*, 251–266. (In Spanish)
28. Chyzik, R.; Ucko, O. Seasonal abundance of the western flower thrips *Frankliniella occidentalis* in the arava valley of Israel. *Phytoparasitica* **2002**, *30*, 335–346. [CrossRef]
29. Tscharntke, T.; Rand, T.A.; Bianchi, F.J.J.A. The landscape context of trophic interactions: Insect spillover across the crop-noncrop interface. *Ann. Zool. Fenn.* **2005**, *42*, 421–432.
30. Fraile, A.; Pagán, I.; Anastasio, G.; Sáez, E.; García-Arenal, F. Rapid genetic diversification and high fitness penalties associated with pathogenicity evolution in a plant virus. *Mol. Biol. Evol.* **2011**, *28*, 1425–1437. [CrossRef] [PubMed]

31. Power, A.G.; Mitchell, C.E. Pathogen spillover in disease epidemics. *Am. Nat.* **2004**, *164*, S79–S89. [CrossRef] [PubMed]
32. Roggero, P.; Ciuffo, M.; Katis, N.; Alioto, D.; Crescenzi, A.; Parrella, G.; Gallitelli, D. Necrotic disease in tomatoes in Greece and south Italy caused by tomato strain of *Parietaria mottle virus*. *J. Plant Pathol.* **2000**. [CrossRef]
33. Marchoux, G.; Parrella, G.; Gebre-Selassie, K.; Gagnalon, P. Identification de deux ilarvirus sur tomate dans le sud de la France. *Phytoma* **1999**, *522*, 53–55. (In French)
34. Aramburu, J. First report of *Parietaria mottle virus* on tomato in Spain. *Plant Dis.* **2001**. [CrossRef]