

Review

A Historical Account of Viruses in Intensive Horticultural Crops in the Spanish Mediterranean Arc: New Challenges for a Sustainable Agriculture

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Abstract: The epidemiological dynamics followed by viruses in protected horticultural crops in the Mediterranean Arc of Spain has evolved from a majority of those transmitted by aphids to the predominance of whitefly-transmitted ones. Later, due to the shift towards an integrated control that has been quite successful in the control of aleyrodids, not so much in the control of aphids, aphid-borne viruses are having a significant revival in open field cultivation. Another threat is the continuous emergence of new species or variants of mechanically transmitted viruses. Thus, the number of viruses affecting these crops is constantly increasing and their control demands dynamic actions. The main measures that have managed to limit the damage of these diseases have been the improvement in the physical barriers that limit the spread of vectors and the introduction of resistances in the germplasm. Recently, the increased movement of plant materials and of people, the popularity of growing local crop varieties that lack natural resistances against pathogens, and the prospects of global climate change, may well have boosted the frequency of diseases and pests. Faced with this picture, strategies must be addressed from a multidisciplinary approach. The need for in-field diagnostics tools, easy access to information, novel breeding technologies and alternatives to control of these viruses are discussed.

Keywords: plant viruses; intensive horticulture; climate change; new technologies; biological control; emergent diseases; decision making

1. Introduction

The climatic conditions of the Mediterranean Arc of Spain, paradigmatic of the Mediterranean basin, make it suitable for intensive and high-quality agriculture, which has led it to become the main supplier of fresh vegetables for European markets [1]. Around 74,300 Ha are dedicated to protected vegetable production in Spain, 89% of which is concentrated in the Mediterranean Arc of the country. This surface duplicates when open field cultivation is considered, although the economical yield is smaller. The economic value of vegetable exports in the country exceeds EUR 4.5 M [2]. Unfortunately, the advantageous agronomic conditions in the area favor the propagation of diverse pests and diseases in vegetable crops, among which we need to highlight the viruses. In this article, we will consider the different virus diseases that have appeared in this area in the last 30 years, their vectors, and the strategies that have been developed for their management, with different degrees of

success, in order that they can help us to face the new viruses that will undoubtedly appear in the coming years.

Viruses in intensive horticultural crops are a major concern for growers in southeastern Spain. Within their order of priorities, these diseases stand out among other factors such as market fluctuations and production costs (Figure 1). This consideration has been maintained over time, but it is important to bear in mind that despite the continuous arrival of new viruses to these areas, production has been maintained and continues to be profitable. However, new risks may arise in the future that could compromise this production model. Certainly, up to now, the joint effort of the different agents involved in the control of these diseases—growers, technicians, researchers and seed companies, in addition to the different actions of the Administration in the form of regulations, assistance and inspections—has managed to limit the harmful effect of these diseases.

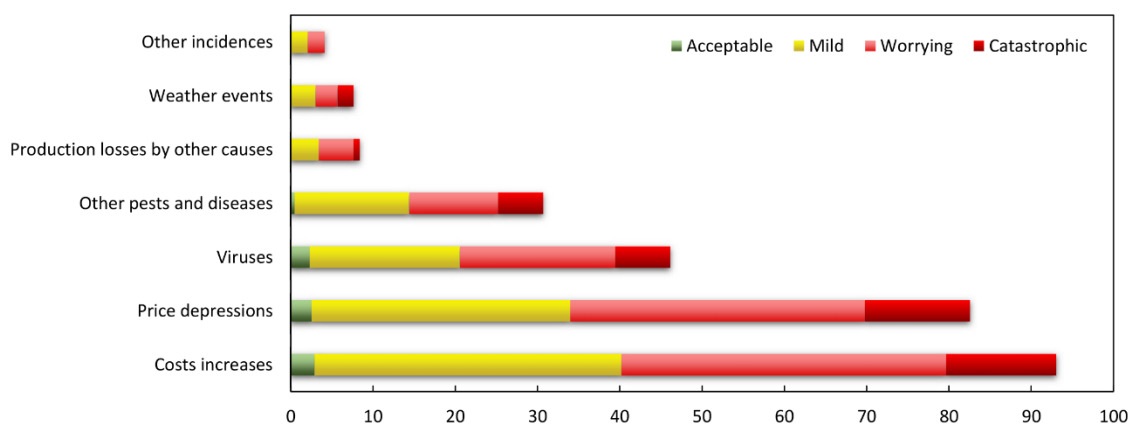


Figure 1. Concerns of Spanish vegetable greenhouse growers according to a survey in Almeria province (elaborated with data from Consejería de Agricultura y Pesca. Junta de Andalucía, 2009) [3].

The evolution of many viral diseases in southeastern Spain has developed in parallel with that of cultivation methods (from open field to greenhouses) and following the emergence or dynamics of existing insect vectors. In this sense, it is worth pointing out as a master line, the appearance in these areas in a first stage of viruses transmitted by aphids, which, although in decline in terms of incidence, have been displaced by the appearance, successively, of different viruses transmitted by whiteflies (Figure 2). We must not forget viruses transmitted by other vectors such as fungi, thrips or those that are propagated by mechanical contact, a growing concern in the present day. A rate of 0.9 viruses per year results from plotting the number of accumulated viruses versus the year of their description (Figure 2) The outbreak of new virus diseases or the overcoming of available resistances in a context of climate change, globalization and IPM production are momentous challenges for vegetable production across the Mediterranean and particularly in Spain, but not unbeatable. In this review, we make a historical account of the appearance, impact and management of the different species of virus in the Spanish Mediterranean arc. In the last section, we present the most important current and future threats to vegetable cultivation in this area. Finally, we propose a series of strategies to address these threats and ensure that agriculture remains both profitable and with minimal environmental impact.

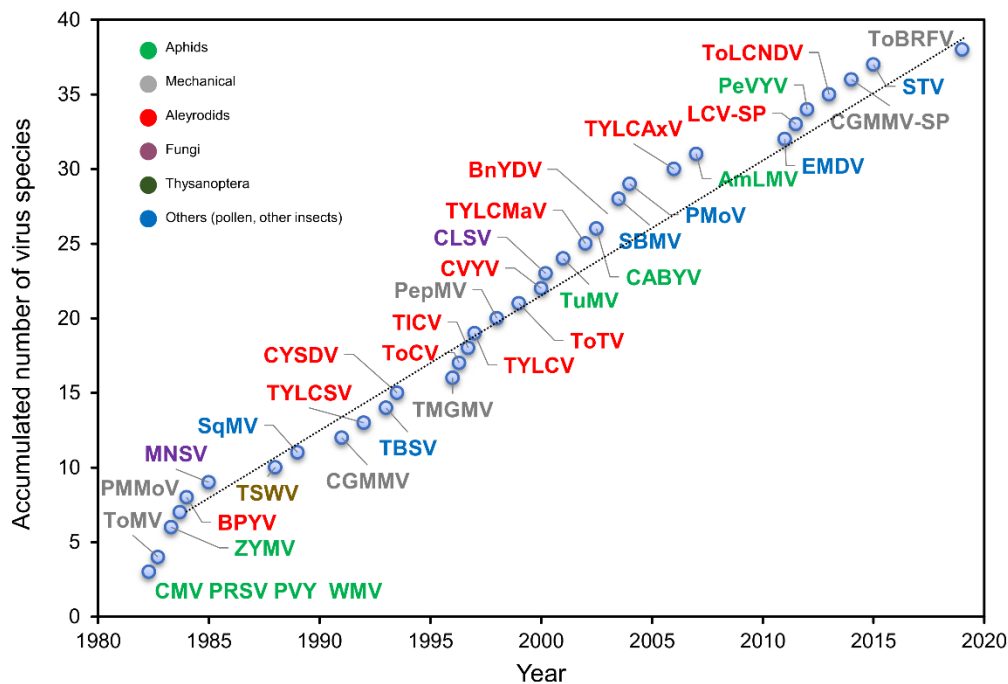


Figure 2. Timeline of accumulated virus occurrences in the Mediterranean Arc of Spain. Virus acronyms: AmLMV (Amaranthus leaf mottle virus), BnYDV (bean yellow disorder virus), BPYV (beet pseudo-yellows virus), CABYV (cucurbit aphid-borne yellows virus), CGMMV (cucumber green mottle mosaic virus), CLSV (cucumber leaf spot virus), CMV (cucumber mosaic virus), CVYV (cucumber vein yellowing virus), CYSDV (cucurbit yellowing stunting disorder virus), EMDV (eggplant mottled dwarf virus), LCV-SP (lettuce chlorosis virus), MNSV (melon necrotic spot virus), PepMV (pepino mosaic virus), PMMoV (pepper mild mottle virus), PMoV (parietaria mottle virus), PRSV (papaya ringspot virus), PVY (potato virus Y), PeVYV (pepper vein yellows virus), SBMV (Southern bean mosaic virus), SqMV (squash mosaic virus), STV (Southern tomato virus), TBSV (tomato bushy stunt virus), TICV (tomato infectious chlorosis virus), TMGMV (tobacco mild green mosaic virus), ToBRFV (tomato brown rugose fruit virus), ToCV (tomato chlorosis virus), ToLCNDV (tomato leaf curl New Delhi virus), ToMV (tomato mosaic virus), ToTV (tomato torrado virus), TSWV (tomato spotted wilt virus), TuMV (turnip mosaic virus), TYLCAxV (tomato yellow leaf curl Axarquia virus), TYLCMaV (tomato yellow leaf curl Malaga virus), TYLCSV (tomato yellow leaf curl Sardinia virus), TYLCV (tomato yellow leaf curl virus), WMV2 (watermelon mosaic virus 2), ZYMV (zucchini yellow mosaic virus). Acronyms' colour indicates the mode of transmission. A linear regression line was calculated for virus accumulation.

2. The Early Years of Intensive Horticulture

In the 1980s, viruses that limited crop production were mainly aphid-borne or mechanically transmitted. Among the aphid-transmitted viruses, cucumber mosaic virus (CMV) stands out because of its polyphagous nature, besides other viruses such as potato virus Y (PVY) in solanaceous crops and papaya ringspot virus (PRSV), watermelon mosaic virus 2 (WMV) and zucchini yellow mosaic virus (ZYMV), affecting cucurbits. At that time, there were also serious epidemics of mechanically transmitted viruses affecting tomato and pepper. The causal agents were mainly the tobamoviruses, tomato mosaic virus (ToMV) and pepper mild mottle virus (PMMoV). All these viruses have been the object of intense research, since they are also important in other parts of the world, which has allowed the introduction of sources of resistance for the different cultivated species. In the case of PVY in pepper or PRSV and ZYMV in cucurbits, the introduced resistances are effective, although not so much for WMV or CMV due to the capability of adaptation of this virus to very different species, which means that it can easily escape from resistances. For the control of ToMV in tomato, the gene *Tm1* introduced at first was later replaced by *Tm2²* [4], which has remained effective against this virus

since the 1950s, until the recent arrival in the Mediterranean basin of tomato brown rugose fruit virus (ToBRFV) [5,6].

PMMoV presents different races that have been appearing successively in the area we are dealing with and constitutes an interesting example of how, as the resistance to this virus is introduced, the selection and displacement of some races by others happens. Resistance in pepper to this virus is provided by alleles of the *L* gene, called *L1* to *L4*, which successively and complementarily controls the races or pathotypes of the PMMoV P0, P1, P12 and P123 and other tobamoviruses [7]. Thus, P0, P1, P12 pathotypes are controlled by the *L3* allele, while P0, P1, P12 and P123 are controlled by *L4*. To date, the most recently introduced *L4* gene has been effective in controlling all PMMoV pathotypes. Even though *L4* breaking P1234 pathotypes have been described in Israel, Japan, and other locations [8,9], they do not seem to be spreading in pepper crops in their respective areas and have not been detected yet in Spain [10]. Most of the pepper cultivars grown in protected horticulture in Almeria province include the *L3* or *L4* resistance genes [11]. Among the viruses transmitted by contact, we must also mention tobacco mild green mosaic virus (TMGMV), another tobamovirus that is pathogenic for *Solanaceae*, in particular pepper and eggplant. TMGMV, in addition to the tobamoviruses ToMV and PaMV, is effectively controlled in pepper by the *L3* and *L4* genes. However, the recent appearance of TBRFV that overcomes *L4* resistance in the Mediterranean basin will certainly compromise pepper cultivation in Spain in the coming years [6]. In addition to genetic resistance, controlling pepper tobamoviruses and other mechanically transmitted viruses requires care in the management of the crop in the case of outbreaks. It is essential to control the working tools, both clothing and footwear, as well as utensils used for different tasks, which can transmit these viruses during the crop cutting and pruning or even because of the movement of specialized workers, including agronomists, from one crop and another. The chemicals preferably chosen for the treatments of tools should be effective in a short contact time, ideally no more than one minute, and safe for workers and the environment [12]. Some growers, however, cultivate traditional varieties, mainly tomato, because of their lower seed price, higher revenues, or that they are more appreciated by consumers. These varieties lack introgressed resistances, and frequently seeds are obtained without disinfection, boosting the propagation of seed-borne viruses.

In addition to these aphid- or mechanically transmitted viruses, two more viruses appeared in this decade, one affecting melon and transmitted by the fungus *Ospidium*, melon necrotic spot virus (MNSV), and another one transmitted by thrips, tomato spotted wilt virus (TSWV). MNSV has been controlled by the introgression of the *nsv* gene in melon [13], which has proved to be effective despite the existence of a recombinant race that overcomes this resistance [14]. Fortunately, this MNSV race has a poor adaptation to the host, so its spread is quite limited [15]. A much more severe disease is the one caused by TSWV, one of the two viruses of genus *Tospovirus* that has entered Spain. This virus, which very polyphagous, mainly affects peppers, tomatoes and beans, and represents a serious disease that has been difficult to manage. TSWV dramatically expanded in the mid-1980s in Spain following the arrival of the Western flower thrips (WTF) *Frankliniella occidentalis*, that displaced *Thrips tabaci* in greenhouses [16,17]. Moreover, the WTF, the only one effective in the Mediterranean basin to transmit that virus, is also polyphagous and difficult to contain because it is very prolific and of small size, capable of trespassing physical barriers in greenhouses. On the other hand, TSWV, being such a polyphagous virus, has many reservoirs, both in cultivated plants and weeds. The displacement by the WTF of *T. tabaci* has been verified in many parts of the world, although not in all environments, as shown by Wu et al., 2020 [18]. Moreover, the rise in CO₂ levels seems to favor the proliferation of the WTF [19], so it is easy to anticipate that the incidence of the viruses it transmits will escalate. In the case of tomato, with the introduction of the gene *Sw-5* from *Lycopersicon peruvianum* [20], despite the appearance of TSWV strains that overcome resistance, these have not spread [21,22] and has allowed researchers to limit the damage to tomato crops provided that the pressure of the inoculum/vector is reduced [23]. In the case of pepper, the situation is more compromised. At first, the introgression of the *Tsw* gene from *Capsicum chinense* in pepper allowed the control of the virus, as long as that the inoculum pressure was moderate, and that the infection was at later stages of plant development or

that plants were not grown under prolonged high temperatures. However, since the beginning of the 2000s, TSWV races that overcome *Tsw* resistance dispersed quickly, causing serious epidemics in pepper. Symptoms caused by these resistance-overcoming races are either the usual TSWV symptoms in pepper varieties that lack the *Tsw* gene, or necrosis and plant collapse in varieties that have the *Tsw* gene. Therefore, the search for new sources of resistance to TSWV in pepper e.g., accession PIM26-1, is crucial [24]. However, this search will have to consider the co-existence of races that overcome the *Tsw* gene and others that do not, as well as the possible interactions that mixed infections may cause in plants [25]. Impatiens necrotic spot virus (INSV), the other tospovirus detected in Spain and transmitted by WTF, seems to be limited in its damage to ornamentals [26].

In the early 1980s, a disease named melon yellows spread in greenhouses in the southeast of Spain and was associated with the greenhouse whitefly (GHW) *Trialeurodes vaporariorum*. Later, this disease was ascribed to a clostero-like virus [27] and it was finally identified as the crinivirus beet pseudo-yellows virus (BPYV) [28]. This virus is not transmitted by the silverleaf whitefly (SLW) *Bemisia tabaci* and, following the displacement of GHW by SLW since the mid 1990s, BPYV has almost disappeared from greenhouses in Spain [28], although it is still frequently found in open field cucurbit orchards [29].

3. The First Major Outbreaks of Whitefly-Transmitted Viruses during the 1990s

Although whiteflies have always been present in Mediterranean agriculture, their importance only relied, until about 30 years ago, on their damage as a pest, being sap-sucking insects. However, since the appearance of BPYV in the late 1980s in Spain, the number of viral species transmitted by aleyrodids has not stopped growing (Figure 2). Tomato yellow leaf curl disease (TYLCD) appeared in Spain in the early 1990s [30]. Two virus species were first identified as causing this serious disease in Spain. They were the begomoviruses tomato yellow leaf curl Sardinia virus (TYLCSV) and tomato yellow leaf curl virus (TYLCV) [31]. The later has displaced TYLCSV plausibly because it is more efficiently vectored by the local biotypes of SLW. In addition, common bean is a host for TYLCV—which causes plant and fruit malformations and dwarfism—but not for TYLCSV [32,33]. Subsequently, two recombinant species of the same viruses have appeared, *Tomato yellow leaf curl Axarquia virus* (TYLCAxV) and *Tomato yellow leaf curl Malaga virus* (TYLCMaV), which have different biological characteristics [34]. Unfortunately, the indigenous biotypes of SLW have been displaced by other more prolific and effective in transmitting viruses, such as the B and Q biotypes [35,36]. These biotypes have shown to have a great capacity to develop resistance to most of the active ingredients on the market, including neonicotinoids and growth regulators. Since then, the biological control of SLW has been the object of intense studies [37–39]. Tomato yellow viruses also infect peppers which are asymptomatic end-point hosts [40].

For the control of TYLCD in tomato, we rely on the gene *Ty-1*, introduced in 1994 from *Solanum chilense*. This gene has been shown to be quite effective when the combined pressure of inoculum/vector is low or moderate, which is not always the case, particularly in open field cultivation [41]. A consequence of the widespread use of the resistance gene in tomato varieties in the southeast of Spain has been the displacement of TYLCSV by TYLCV [11], which offers better adaptation and higher multiplication rate in plants bearing the *Ty-1* gene [42]. As mentioned above, the appearance of recombinants may be another consequence of the extended cultivation of resistant tomato cultivars [42,43]. Other TYLCD resistance genes have been identified in different members of genus *Solanum* include *Ty-2*, *Ty-3*, *Ty-4*, *Ty-5* and *Ty-6*, which are not yet available in commercial varieties [44–46]. *Ty-1* and *Ty-3* are alleles of the same gene and could provide, used in combination, alternative resistance to TYLCD [47]. These genes could pyramidally complement the *Ty-1* resistance and even lessen the possible damages that the eventual introduction of other begomoviruses would cause in our growing areas [48]. Another source of resistance to TYLCD, not related to the *Ty* genes and recessive, has been recently found in a tomato accession capable of limiting the primary spread of TYLCV [49]. Other complementary alternatives that are currently being developed are to improve

resistance to the vector through the introgression of genes that increase trichomes in the leaves or that produce substances that repel vectors or induce resistance in the plant [50,51].

Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus that affects cucurbits, particularly cucumber but also melon and watermelon [52]. It was detected in the area in the mid-1990s [53] and is a characteristic case of a virus that has sporadic, sometimes severe outbreaks. Thus, it is very important to recognize the symptoms and get specific diagnostics in order to stop its appearance in the crop [54]. Recently, a phylogenetically distinct race appeared in the region similar to the Asiatic variants of this virus [55]. This race is more prolific than the previous one, the European variant, and is causing frequent and severe outbreaks in cucurbit crops in Spain. There is currently no resistance to this virus in commercial varieties, although research is in progress [56]. Tobamoviruses such as CGMMV, ToMV or PMMoV are seed transmitted. Debris of infected plants after crop removal in the field remains infective after long periods. For this reason, when there is an emergence of a tobamovirus or any other mechanically transmitted virus, among the measures taken by the authorities of removing any residue, a quarantine must be imposed on the field and restrictions to grow susceptible plants to the virus should be made effective. Particular care must be taken when plants are managed not only in the greenhouse, but, in addition, in the nurseries, where cucurbits are frequently grafted for seedlings preparation [57].

Pepino mosaic virus (PepMV; genus *Potexvirus*) is one of the most severe mechanically transmitted viruses in Spain [58]. Despite its name, it does not affect cucumber but tomato, as it is derived from the solanaceous south American plant sweet cucumber (*S. muricatum*) [59]. This virus appeared in Spain at the end of the 1990s and since then it has been one of the most serious epidemics in tomato in all the Mediterranean Arc of Spain [60]. There are not yet resistances to this virus in commercial varieties of tomato, although it is a work in progress by public and private entities [61]. In the meantime, it is necessary to control the virus by prevention through strict sanitation measures, as for the other mechanically transmitted viruses. Cross-protection by the immunization of crops with low-virulence PepMV strains that protect against the more aggressive strains has been used in Spanish greenhouses. It is a viable method but requires highly controlled conditions and well-defined virus genotypes to be available [62]. PepMV epidemics in intensive tomato crops in Spain are caused by both CH2 and EU isolates that co-circulate. Mixed infections with two mild isolates of the EU and CH2 strains (PepMV-Sp13 and -PS5, respectively) have recently been proposed for use in PepMV cross-protection, providing protection against a broad range of aggressive isolates [63–65].

4. The Emergence of Existing and New Virus Species during the 2000s

An outbreak of the ipomovirus cucumber vein yellowing virus (CVYV) happened in the southeast of Spain at the end of 1999, causing a dramatic impact on cucurbit yield and fruit quality [66]. Some years before, the crinivirus cucurbit yellowing stunting disorder virus (CYSDV) made its appearance in cucurbit crops [28]. Both are transmitted by the SLW *B. tabaci*. It is worth mentioning that BPYV has hardly been detected lately in Almería or Granada provinces, no doubt because its vector *T. vaporariorum* has been displaced in the area by SLW [28]. On the contrary, CYSDV and CVYV are species of special relevance in intensive crops in southeastern Spain. The control measures for CYSDV has been, on one hand, the introduction of resistances in cucumber [67] and melon [68] and on the other hand, the control of the vector, mainly at the level of physical barriers in greenhouses: double doors, high density nets, etc. [69]. Physical barriers have been effective for the control of CVYV as well [69]. In addition, the introgression of a resistance gene in cucumber has been shown to be efficient in reducing susceptibility to mild variants of the virus, but not to severe ones that are not currently present in the area [70–72]. The resistance to CVYV in cucumber has been recently mapped in a locus called *CsCvy-1* [73]. In melon, there are some advances in the mapping and introgression of the resistance, due to the fact that it is more complex than in cucumber, because three loci have been found to be controlling resistance and symptom severity [74]. In watermelon, there are no reports of resistance to CVYV, although some seed companies are dealing with this trait. CVYV also affects

watermelon, causing internal necrosis in the fruit flesh that is undetectable until the watermelons are opened. However, sometimes the damage of this virus to the fruit is so severe that it eventually develops cracking.

Cucurbit aphid-borne yellows virus (CABYV) was first identified in open-field melon crops in Campo de Cartagena (Murcia region) and later it was detected in adjacent provinces [29,75]. It is an aphid-borne polerovirus that causes symptoms in different species of cucurbits. These are variable, ranging from mild yellowing to complete discoloration of the plant and the thickening of leaves, which become brittle. It does not affect the quality of the fruit, although it does reduce production. Due to the way the virus is transmitted, in principle it is more common in outdoor cultivation areas, the most common way of growing melon in the region of Murcia [29]. However, as we have mentioned before, the greater appearance of aphids, even in winter areas, means that it is sporadically detected in greenhouses in the province of Almeria. CABYV control in melon is feasible by the introgression of the two recessive genes *cab1* and *cab2* [76]. Another promising source has been described in melon accession TGR-1551 [77]. In other cucurbits, resistances are also available [78]. It is expected that the incidence of aphid-borne viruses will increase until the integrated control of these insects is effective, if ever. Control of aphid-borne viruses improves when aphid resistance genes are introgressed, such as the *Vat* gene for melon, which confers some resistance to *Aphis gossypii* [79].

Another virus that we must consider is one transmitted by the fungus *Olpidium*, as is the case of cucumber leaf spot virus (CLSV) [80]. This virus has been occasionally detected in cucumber crops and its importance seems to be minor. This is not the case of pepper vein yellows virus (PeVYV), a polerovirus that appeared in 2012 in pepper greenhouses in Spain [81]. The damages caused by this virus are mainly the irregular ripening of the fruit, malformations and discolorations in those varieties in which there is a change of color. This implies a loss of the commercial value of the fruit, which is critical in the high-end standards demanded for vegetables' appearance in the EU markets [82]. PeVYV is paradigmatic among viruses transmitted by aphids in southeastern Spain. The control of whiteflies by conventional methods, i.e., pesticides, had as a collateral consequence in the significant reduction in the incidence of aphids and consequently of the viruses transmitted by them. The move towards integrated control methods has resulted in an effective control of whitefly incidence, which has not been the case for aphids. This increase, however, has not been translated into a generalized presence of aphid-borne diseases except in the case of zucchini and peppers crops, where this pest has become more relevant. For example, in zucchini, there has been an increase in CABYV incidence (www.juntadeandalucia.es/agriculturapescaydesarrollorural/raif/), and this may also explain the emergence of PeVYV.

The ilarvirus parietaria mottle virus (PMoV) was detected in pepper greenhouses in 2004 [83] and next in tomato [84]. The symptoms in these crops resemble those of TSWV, such as discoloration and necrosis symptoms in leaves and fruits. Its mode of transmission seems to be pollen, although other insects like thrips could also be involved, in addition to the mirids used for biological control, in a nonpersistent manner [85–87]. After PMoV outbreaks in tomato and pepper, only a few small PMoV infection foci have been reported, often linked to mirid release for biological control. Isolates of PMoV detected in tomato crops in Catalonia region seem to be genetically indistinguishable from the isolates of pepper from Almeria [85].

Another crinivirus, tomato chlorosis virus (ToCV), transmitted by both the SLW and GHW, was introduced in southeastern Spain in the late 1990s. This virus produces yellowing of tomato leaves and reduces the photosynthetic capacity of the plant, therefore decreasing yield, although it does not affect fruit quality [88]. It also infects pepper, causing important losses [89]. A related species, tomato infectious chlorosis virus (TICV), with similar characteristics to ToCV has been sporadically detected in the southeast of Spain; however, as it is only transmitted by *T. vaporariorum*, its incidence is very low [90]. Yellowing caused by these viruses is easily mistaken by farmers with plant nutrient deficiencies or aging. This is particularly evident in areas where tomato crops are under nets where vectors easily enter and transmit the disease. These symptoms, in contrast, are hardly observed in tomato crops

under strict vector control measures [90]. There are currently no commercial tomato varieties with resistance to these viruses, probably because it is not claimed by farmers to seed companies due to the lack of advising on this disease.

Ongoing with tomato viruses transmitted by whiteflies (SLW and GHW), we need to mention tomato torrado virus (ToTV), a species that has given its name to the genus *Torradovirus* [91]. This virus caused controversy on its outbreak in the Murcia region because its mode of transmission was not clear. It induces very characteristic symptoms in tomato, both in fruits and in the plant. On the other hand, accidentally, it happened that a recessive resistance to the virus was already present in some commercial cultivars of tomato. Markers are now available for this source of resistance in tomato [92]. It is possible that this already present resistance, together with the low transmission efficiency by the vector, was the reason for the low spread of this virus and the regression that has been observed in some areas of tomato cultivation [17,58].

Other minor crops, such as beans, are also targets for certain viruses. Bean and other legumes are grown under greenhouses in the southeast of Spain and are destined for the fresh market. In 2002–2005, a survey was conducted in order to quantify the incidence of viruses that limit yield and quality of the production [93]. The most frequent viruses were shown to be TSWV, TYLCV, bean yellow disorder virus (BnYDV) and Southern bean mosaic virus (SBMV). Aphid-borne viruses such as bean common mosaic virus (BCMV) and bean common mild mosaic virus (BCMNV) were almost absent, probably because of the limited presence of aphids in legume greenhouses. BnYDV, a novel crinivirus, was identified and first described in 2003 in *Phaseolus vulgaris* in Almeria [94]. The symptoms that this virus causes in beans include yellowing and malformations in leaves and fruits. Since its discovery, it has been observed that BnYDV incidence has been in regression in parallel with the decrease in the cultivated area of beans in the provinces of Almería and Granada [95]. Furthermore, in the last years, this virus has been displaced in bean greenhouses by a novel emergent recombinant of lettuce chlorosis virus (LCV-SP) and BnYDV (see below).

The most effective control measures, in a first stage, for limiting whitefly-transmitted viruses have proved to be the physical barriers. By definition, the greenhouse structure forms a very important physical barrier for preventing the entry of pest insects and virus vectors. The mobility of two main virus vectors in southeast of Spain, *B. tabaci* and *F. occidentalis* depends on the presence of ultraviolet radiation from sunlight. Consequently, reducing this radiation through the use of UV-absorbing plastic limits insect mobility [96]. Also, a wide range of anti-insect meshes are nowadays available for the roof and side windows of greenhouses. Window screens can have meshes of different photoselectivity and density, as well as various colors, but high densities can compromise crop ventilation. So, it is crucial to balance the efficacy of the mesh in excluding insects with adequate permeability for the free passage of air. Finding the correct mesh for greenhouses and crops is a complex task and requires continuous research and development [97]. In the southeast of Spain, for tomato, TYLCD and ToCD were dramatically reduced when improving the quality of the physical barriers that limited insect entry in the greenhouse [90]. UV-absorbing plastic covers that disturb insect visual orientation in the crop have demonstrated efficiency in limiting TYLCD spread in tomato greenhouses [98]. Records across the Mediterranean area are in alignment with these results [99]. In a similar way, CVYV and CYSDV epidemics decreased in well-isolated greenhouses [69].

Multiple infections are common in open field-grown vegetables [100], but not so in greenhouses, where plants are usually infected with a single virus species and the frequency of multiple infections is low, even in low quality greenhouses, such as those of beans [93]. Nevertheless, a factor to take into consideration is the interactions by simultaneous infection of different viral species, which sometimes cause synergisms, leading to the appearance of damage that they would not cause separately. In Spanish greenhouses, CYSDV and CVYV do not cause symptoms in zucchini during single infections, but they cause yellowing and malformations in the leaves, and decrease production yield when they simultaneously infect the plant [101]. An example of the opposite situation is the antagonistic interaction between CGMMV and ToLCNDV in zucchini and cucumber [102]. During mixed infections,

viral ToLCNDV titers are reduced in cucumber and zucchini, and fewer zucchini plants express the typical symptoms associated with the begomovirus. One consequence of this interaction is that co-infected plants might look healthy and it may not be considered necessary to be removed from the crop, thus acting as sources of inoculum. In pepper, it has been reported that there is a striking synergism between *Tsw* resistance-breaking and non-breaking isolates of TSWV. The presence of the first type of isolate enables the infection by the second one, triggering a systemic necrosis reaction in the TSWV-infected pepper plants [25]. Another example of antagonism in horticulture species constitutes potato spindle tuber viroid (PSTVd) (family *Pospiviroidae*) that reduces infectivity and titers of TYLCSV in tomato plants [103]. Finally, in addition to the example described above for the use of antagonism between PepMV strains in cross protection, before the introgression of the *Tm* resistance genes, mild strains of TMV were successfully used to protect tomato plants against the infection by severe strains of TMV and/or ToMV [104].

5. New Virus Species that Emerged from 2010s until Now

This decade started with an outbreak of tomato leaf curl New Delhi virus (ToLCNDV), a begomovirus transmitted by the SLW that appeared in 2012 in southeastern Spain [105,106]. Since its outbreak, this virus led to the practical impossibility to grow open field zucchini in the area, as was done as a common practice in coastal areas of the provinces of Granada and Málaga. This crop, that up to that moment was mostly unaffected by other cucurbit viruses such as CVYV, CYSDV and others, used to be cultivated under low quality covers or even open field. The ToLCNDV isolate present in Spain mainly affects cucurbits, causing serious damages in zucchini, but also, although to a lesser extent, in cucumber and melon. The most probable reason for the lower impact on melon and cucumber crops may be found in the best structures, in terms of physical barriers to the vector, under which these two vegetables are grown, unlike zucchini. This virus also causes mild symptoms in tomato that, nevertheless, is an effective host for this virus and should be considered in management programs [107]. The Spanish ToLCNDV isolate lacks a betasatellite, probably explaining the mild symptoms that it causes in tomato [106]. However, ToLCNDV variants that include betasatellites are frequent in other parts of the world and it is hard to predict what would happen if one of these isolates carrying betasatellites appears in the region. In the meantime, ToLCNDV has moved to other parts of the Mediterranean basin [108]. Intense research is currently underway by public and private entities that will eventually lead to the introduction of resistance to this disease in zucchini and the other cucurbit species [109–111]. In the meantime, control measures for this disease must target the vector.

Southern tomato virus (STV) was first detected in Spanish tomato crops from the Canary Islands in 2015 [112]. It was associated with ripening and color alterations in tomato fruits; although it has been shown that this virus does not induce ultra-structural tissue changes, it alters the accumulation of some plant miRNAs involved in multiple cellular processes [113]. STV is a persistent double-stranded RNA virus from genus *Amalgavirus* (family *Amalgaviridae*), transmitted only vertically by seeds at rates of up to 80%. It is widely spread in the southeast of Spain and Canary Islands, where STV has been frequently detected in mixed infection with other pathogenic viruses such as ToMV, CMV or PePMV [114]. Preliminary studies suggested complex interactions of STV with some of these pathogenic viruses, producing alterations in symptoms and small RNA populations of infected tomato plants (Galipienso, unpublished).

Finally, recombination between two crinivirus species that share the same area of distribution led to the appearance of a new virus disease [115]. This is the case of the recombination between the BnYDV, previously detected in bean crops in Southeastern Spain [94] and LCV, a crinivirus that infects lettuce but not bean, that led to the appearance of the new recombinant strain LCV-SP, capable of infecting bean. The function of the putative P26 and P6 (shared by LCV-SP and BnYDV) are unknown but could be associated with the ability of the recombinant to infect a new host [95,116].

6. Clues for the Future Control of Plant Viruses in Mediterranean Environments

The past always gives clues about the future, although it is true that there may be unpredictable events [117]; general trends in climate and globalization impact have been quantified and the need to address them represents a challenge for plant pathology. In the last few years, a number of new tools and developments have gone from exciting promises to tangible results that are accelerating and facilitating the control of plant diseases caused by viruses and their vectors. These tools stand out for their multidisciplinary nature and involve collaboration between different areas of expertise, including breeders, entomologists, agronomists, pathologists, bioinformaticians, government officers and growers. In our opinion, the following are the most significant hints for the present and future control of plant viruses in the Mediterranean basin and in Mediterranean-like environments.

6.1. Shift to Biological and IPM Control

Pesticide residues are the most important food-related concern for EU consumers according to Eurobarometer [118]. Furthermore, pesticides for pest control are progressively ineffective because of the appearance of resistances [119], and more importantly, the strict limitations in application of chemicals for insect control in the EU (Arthropod Pesticide Resistance Database, APRD, <http://www.pesticideresistance.org/>; European Commission—Pesticides Database, <http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database>). Another handicap of the use of pesticides is the unspecific harm to the native fauna that control pest populations. Together with regulations, all this has led to expanding the use of integrated pest management (IPM) and biological control in vegetable production in the EU, and particularly in southeastern Spain, that currently leads the biological control application in the world [120]. Nevertheless, the application of solely biological control for pests and virus control is difficult, and there is growing evidence that IPM, instead, would be the most effective alternative in the balance of profitability and reduction in environmental damage in vegetable cultivation in the Mediterranean Arc in Spain. The use of pesticides to control whitefly-borne viruses is limited to the minimum, and preference is given to alternative approaches that combine biological, biotechnological, and chemical means, as well as cultural practices and plant breeding. All together, they make up IPM, in which these control measures must be combined intelligently in order to keep the population levels of arthropod, pests and virus vectors below their economic damage thresholds. In contrast to temperate climate regions, Mediterranean countries have a special problem regarding insect control, since there is a higher incidence of virus diseases and pests. The situation regarding the levels of phytosanitary product residues on horticultural produce in Almeria has improved dramatically in recent years. In 2007, following the problems of pesticide residues detected on exported produce and the increase in resistance to pesticides among pests, the use of biological control became widespread and the key was integrated pest management [97]. Today, most of the greenhouses in Almeria use biological pest control, and have turned it into the largest concentration of greenhouses in the world to use biological pest control [121]. IPM and biological control has now become an alternative approach for the management of whitefly-borne viruses in Mediterranean greenhouses [122].

In a recent report, integrated management was revealed as the best strategy to control ToLCDV spread in greenhouse-grown zucchini, followed by biological and chemical control, in this order [123]. Nevertheless, efforts to move to biological controls are being taken into consideration because of public concerns [124]. In addition, the use of insect predators in biological control has been prone to indirect benefits. So, the zoophytophagous predator *Nesidiocoris tenuis* used for the control of *B. tabaci* and other pests is capable of inducing plant defenses in tomato due to its phytophagous behavior [125]. Moreover, it has been observed that the frequent liberation of predators or parasitoids by the growers or government officials has dramatically reduced the presence of pests in the area [126]. However, it is important to point out that the control of insect-borne viruses by means of IPM or biological control is effective solely in protected crops, being almost unfeasible in open field cultivation. In addition, open field grown vegetables show high rates of aphid- and whitefly-borne virus infection, including frequent multiple infections [100], making them difficult to manage. Under these conditions, the

symptoms are difficult to interpret and the lack of practical methods of diagnosis in situ, unawareness of the viruses present and their mode of transmission, and confusion as to the resistance available in the plant material, lead to the failure of the crop.

Aphid control using insecticides led to widespread resistance against many active compounds. In addition, environmental and consumer concerns imposed stringent regulations on residue levels and the move to IPM. However, biological insecticides are scarce and show, in general, low efficacy. Aphid predators and parasitoids are difficult to manage and, consequently, biological control alone provides poor efficiency and growers end up using chemicals compatible with natural enemies whenever possible [127]. Moreover, aphids are efficient virus transmitters even at low populations [128]. Not surprisingly, the incidence of aphid-borne viruses has intensified in the last few years in Spain. Among the IPM approaches proposed, production under UV-absorbing covers has been reported to reduce the aphid-borne lettuce mosaic virus infestations in lettuce [129].

6.2. Effects of Climate Change on Viruses and Vectors

Both temperatures and atmospheric CO₂ concentrations are expected to increase over the current century, especially in the Mediterranean [130]. Although there are quantitative models that foresee different rates of growth in these two parameters, which are the ones that most affect the so-called climate change, there is no doubt that they will have consequences on the incidence of plant pests and diseases (reviewed in: [131,132]). Still, knowledge on the combined effect of rising temperatures and increase in CO₂ levels in plant hormonal balance and defense systems in the different pathosystems remains scarce [133]. On the pathogen side, changes in mutation rate and virulence, are also affected by environmental conditions (reviewed in: [134]). In the specific case of plant viruses, two main factors are considered to deserve investigation: (i) plausible rising in the severity of virus epidemics and (ii) growing difficulties in controlling them [135]. According to numerous studies, Spain is one of the countries most exposed to climate change [136,137] (Figure 3), and several models predict that CO₂ levels will duplicate by 2050 in the country [137], so it is essential to take these phenomena into account in any scheme of future projection of the incidence of diseases and pests on crops.

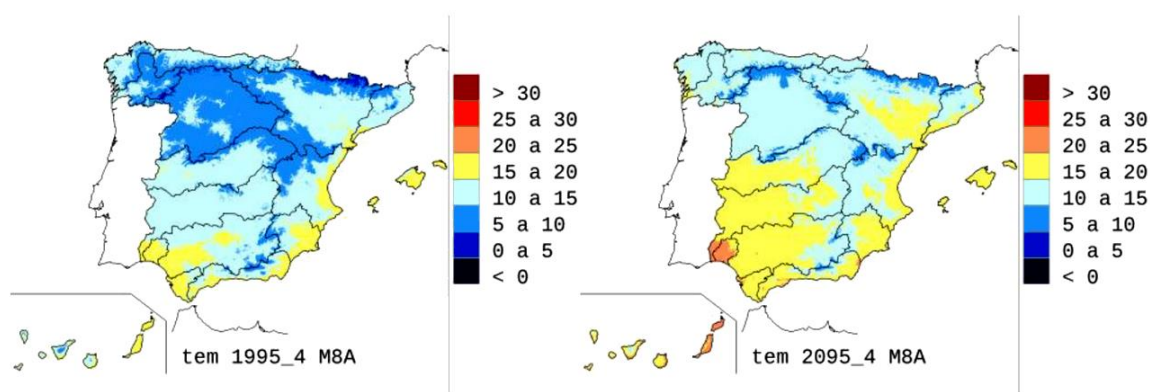


Figure 3. Projections of change in daily mean temperature (°C) from April 1995 to April 2095 in Spain (Source: CEDEX, 2017 [137]).

Like many plant species, horticultural crops require temperatures above 10–12 °C in order to grow and to produce adequately. Temperature levels leading to maximum production are between 16 and 20 °C during the night and between 22 and 30 °C during the day [138]. Many arthropods vectoring viruses also have their optimal development at this temperature range, so all strategies to control the climate in greenhouses using energy-saving screens, heating systems, etc. will eventually determine the spread of viruses [97]. Improving climate control technology and gaining detailed knowledge on the effects of temperature, humidity and light on plant viruses and their vectors will lead to better virus control. These factors will become even more relevant when considering prospects of climate

changes, when vectors spread and are installed in more geographic regions [139]. Likewise significant technological and management changes that lead to whole year-round production of greenhouse crops, such as those introduced by greenhouse farmers in northern temperate climates with the use of continuous or extended lighting, may have effects on virus vectors that need to be understood and could represent new challenges for virus control [140].

However, when considering the effect of temperatures in vector-borne disease, it is important to distinguish between the rise in average temperatures and appearance of extreme temperature events. There are scarce reports in the literature on the effect of temperatures on virus spread in Spain, but in other areas, abundant information is provided. Ruiz and col. observed that CVYV epidemics increased with temperature in cucumber crops [69], while CYSDV spread was not affected. In most cases, plants develop hypersensitive responses to virus infection at low temperatures but are systemically infected at high temperature [141–144]. For example, it has been reported that high temperatures reduce PepMV symptoms in tomato [145]. In consequence, PepMV incidence can be underestimated and its unintended dispersion increase. High temperatures in growth chambers reduce three-fold the time necessary for PVY infection to become systemic in potato plants [146]. In the case of tobamovirus resistance in pepper, it has been reported and commonly observed in the field that *L* alleles lose efficiency above 30 °C [147]. Reports on TSWV resistance have shown that high temperatures (>30 °C) and, more importantly, continuous high temperatures compromise resistance in pepper cultivars, resulting in plant systemic infection [148,149]. Regarding non-specific resistance, it has been repeatedly observed that RNA silencing is more efficient at high temperatures [150–152]. Another consequence of temperature rise is the emergence of resistance breaking variants because of diurnal temperature variations. The inactivation of resistance when the temperature is high would allow viral replication, generating a population of mutants. The activation of resistance when the temperature decreases would allow for the selection of resistance-breaking mutants [153]. Examples can be found in the literature of opposite effects between temperature rise and virus susceptibility, including those described above. For example, high temperatures limit symptoms in potato triggered by most of potato virus X (PVX) isolates [154]. MNSV symptoms are more frequently observed in protected crops in the period from autumn to spring, probably due to reduced symptom expression at high temperatures [155].

From the vector side, low temperatures decrease insect activity, thus restraining virus epidemics. In open field cultivation, winter temperatures limit WMV spread, indicative of cold sensitive vectors [156]. *A. gossypii* has shown considerably higher mortality during development at both low and high temperatures [157]. Regarding CO₂, it has experimentally been shown that high concentrations and elevated temperatures impair synergism in multiple virus infections. Elevated CO₂ upheld the synergistic interaction of potyviruses in *Nicotiana benthamiana*, whereas higher temperatures decreased virulence and virus titers [158]. While there is a general trend of increased susceptibility to viruses and vector activity with temperature rise, no generalizations can be made. The evolution of each pathosystem under these conditions is unique and depends on the nature of the virus and its vector as well as the characteristics of the sources of resistance. Finally, increasing temperatures have already led to the poleward movements of pests and pathogens at a rate that has been measured in what has been called the global warming-driven pest movement [159]. Although virus movement has shown, on the contrary, an equatorial shift, this has been explained in terms of biases in diagnostic availability (Figure 4). Another plausible explanation is that the origin of the viruses in many crops must be in the same place as the centers of origin of the crops' ancestors, which are usually, at least, in Eurasia, the temperate regions. From here they will move to other parts of the globe. In any case, with the arrival of new insect species in temperate regions, both new virus species and new vectors for already established viruses can lead to challenging situations.

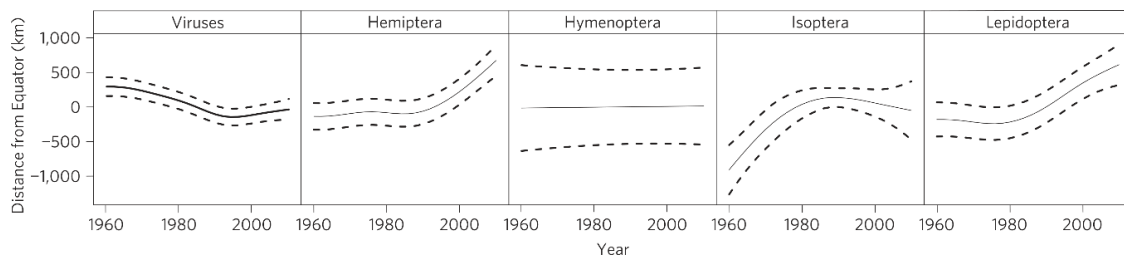


Figure 4. Poleward movements against the year of observation of plant viruses and different insect families that include relevant vectors. Redrawn with permission from [159].

6.3. Resistance Breeding in a Climate Change Context

In nature, there is coevolution or an evolutionary arms race between hosts and pathogens, since pathogens impose selective constraints, causing the evolution of resistance characteristics that reduce the survival or virulence of viruses [160,161]. This imposes selective constraints on the virus to evolve characteristics to overcome the plant resistance and, in response, plants must evolve other traits to counterattack and so on. As an illustration, RNA silencing is a basic defensive mechanism against viruses in plants [162]. Viruses counteract by evolving proteins interfering with RNA silencing (viral suppressors of RNA silencing, VSRs) [163]. Plants evolved resistance genes coding proteins to recognize VSRs' essential motifs (avirulence determinants, AVR) to trigger the hypersensitive response and restricting virus movement [164]. To cope with the rapid evolution of pathogens, resistance genes can undergo rapid evolution not only by punctual mutations but by duplications and recombination between homologous genes to generate new specificities [161]. Viral infections have been shown to increase the frequency of homologous recombination [165], which could be a plant defense mechanism to develop new recognition specificities. The frequency of the appearance of recombinants proliferate under the selective pressure of resistance genes, as described above or the sharing of hosts of different virus species [42,43,95].

Viruses can escape plant perception by mutations in the viral AVR [153,166]. However, in some cases, these mutations cannot be fixed in the viral population as they are subjected to strong negative selection or are associated with fitness loss in other hosts or in other functions of the AVR protein. In addition, plant resistance supposes a cost and has evolved to be inducible to save resources for plant growth and development under pathogen-free conditions. In agricultural systems, "artificial" coevolution occurs between the emergence of new viruses or viral variants and breeders implementing new resistance sources in crops [160]. Another point to consider is the jumping of virus species from wild flora to cultivated plants. For example, viruses such as the potyvirus *Amaranthus* leaf mottle virus (AmLMV), which was detected in the weed *Amaranthus viridis* but is not capable of infecting crops, could eventually develop mutations or recombinations that alter their infectivity and range of hosts [167]. Identifying viruses in weeds growing near cultivated plants using conventional methods is limited [39].

Breeding resistant cultivars is a lengthy and costly process. Most resistances implemented in breeding programs are monogenic based on dominant alleles encoding proteins that recognize specifically a viral sequence or conformational pattern (AVR) and induces death of the infected cells (hypersensitive response), restricting viral movement [168,169]. However, the emergence of viral variants overcoming or breaking this type of resistance is frequent, so predicting the resistance durability is one main concern for breeders. Several models have been proposed based on the evolutionary potential of viruses [170] such as (i) the effective population size of viruses to infect a resistant host, (ii) the frequency of recombination and gene flow [171], (iii) selective constraints to resistance-breaking mutations [172] and (iv) the opportunity of the resistance-breaking mutations to occur and the associated fitness costs [173]. Likewise, experimental approaches can be used to evaluate resistance durability based on serial passages on the resistant host and estimating changes in the fitness on viral infectivity and/or accumulation) and symptom severity [174,175]. However, so far,

a systematic prediction of the durability of new resistances remains elusive and it can only be tested on the field. An alternative is breeding quantitative trait loci (QTLs) conferring more durable resistance, as more mutations are necessary for resistance breakdown. However, this type of resistance is more difficult to introgress in crops and cannot prevent virus infection (reduces viral accumulation and disease severity) and its efficiency is more dependent on the genetic background and environment than monogenetic dominant resistances [153].

Considerable progress has been made in the last few years in the identification of the genetic determinants of resistance to many viruses affecting vegetable crops grown in the Mediterranean basin [13,20,48,176,177]. These findings are useful not only for the development of markers in breeding programs but may eventually serve to obtain new allelic sources of resistance. The new developments in genetic editing can facilitate this approach [178]. However, in Europe, consumers and citizens are reluctant to accept the cultivation of genetically modified (GM) crops. This has led, unfortunately, to the decision made by European Union Court of subject CRISPR plants to strict GM laws [179]. Hopefully, it is not entirely out of the question that in a second instance in Europe, the steps taken by legislators in Australia or the USA will be followed [180]. In the meantime, breeding for genetic resistance in our crops must rely only on conventional methods that can be assisted by non-destructive tools such as high-throughput phenotyping [181].

Finally, given that resistance breeding programs take years to accomplish, and do not necessarily culminate in success, it is crucial to think of alternatives. Virus-derived dsRNAs have been proposed as valuable tools for plant virus control inducing the RNAi mechanisms [182]. Different *in vivo* and *in vitro* dsRNA production methods have been described [183] and their topical application on the plant has been reasonably successful in protecting against plant viruses [184,185]. More recently, it has been shown that the release of nanoparticle-protected dsRNAs is more effective than naked dsRNAs for artificial induction of RNAi resistance in plants [186,187].

6.4. New Diagnostic Tools

Growers and agronomists demand fast, cheap and *in situ* detection tools in order to make effective and immediate decisions in the field. Although strips based on antisera for the detection of a few plant viruses are commercially available, experience demonstrates that the results are not always reliable. On the other hand, methods based on nucleic acids amplification such as PCR require sophisticated equipment. The so-called point-of-care testing assays of plant pathogens do not require sophisticated equipment and can be carried out by a non-specialist out of the lab and open a new window of opportunities [188]. The isothermal amplification of nucleic acids (e.g., loop-mediated isothermal amplification, rolling circle amplification, helicase dependent amplification or recombinase polymerase amplification) is prone to being adapted to *in-field* diagnostics with basic devices [189–191]. Fluorimeters are used in the lab for the detection of nucleic acid amplification, but in order to be managed by any user and condition, the detection of amplification should be made with unsophisticated methods. Among the alternative effective amplification visualization methods, flocculation of nucleic acids with nanoparticles must be taken into account [192].

Next generation sequencing (NGS) is probably one of the most powerful tools for plant pathogen detection [193]. However, these tools are, in general, of exclusive use in research laboratories. Recently, however, NGS technologies have been adapted to *in-field* diagnostics of plant viruses. Thus, Oxford Nanopore Technologies has developed the portable MinION sequencer. This device is the size of a smartphone, is USB powered, and can stream up to 100 MB of sequence data over a 24 h period. Nanopore sequencing is based on single molecules in real-time by translocating the DNA through a nanopore (in membrane proteins or synthetic materials such as silicon nitride and aluminum oxide) through which an ionic current passes under an applied voltage. The DNA passing through the nanopore changes the current depending on the shape, size and length of the DNA sequence. This technique allows for a rapid sample processing, without the need for reverse transcription for RNA viruses [194]. The MinION sequencer has been used for the detection of plant viruses in the

field [195,196], but it is still too expensive for most routine uses. It is expected that further development of this promising technology will allow the field-monitoring of plant viral diseases in a near future.

6.5. Epidemiology and Artificial Intelligence in Decision Making

In our experience and others [197], up to now greater risks for vegetable crops in Spain have come from the entry of pests and pathogens from abroad rather than from climate events. The risk increases when the centers of genetic diversity of some pathogens, in some cases even more virulent than those already present, are in the same climatic zone and there is a frequent exchange of goods. CVYV represents a good example of this type of threat [71,72]. The control of the entry of plant material in any form (seeds, cuttings, whole plants) is seemingly regulated and subject to the traceability required by the phytosanitary or plant passport in the EU (https://ec.europa.eu/food/plant/plant_health_biosecurity/non_eu_trade_en). In practice, it is impossible to control the entry of all plant material coming from abroad, not only because the borders of the EU have many doors of entrance, but also because in many sea and airports the control is quite lax. Furthermore, the number of inspection agents at the borders is very low and their ability to detect pathogens or pests in plant material is very limited with the tools available, relying mostly on visual inspection. Thus, it is not possible to control the entry of all plant material coming from abroad. This is in contrast with the strict regulations and effective control of plant material entry in countries such as Australia.

Plant pathogen detection could benefit from the use of nanosensors capable of nondestructive, minimally invasive, and real-time analysis [198]. The use of sensors to capture live images, artificial intelligence to identify and count arthropod vectors in greenhouses, mathematical models to predict their evolution, and decision support systems to assist the farmer on when and how to apply treatments will become increasingly important for modern greenhouse horticulture [199]. On the other hand, the ubiquitous availability of mobile devices can and should make it easier for users to access accurate and up-to-date information on pests and diseases which would allow for quick decision-making in the field and allows collaboration among different agents. Several mobile applications are available for pathogen identification in cultivated plants [12]. Evidence-based decision making requires precise pathogen identification to gather information on disease incidence [200]. Quick and accurate disease monitoring would allow not only growers to make reliable decisions on their crops, but it would also be invaluable in the quantification of the disease in a given area in order to make estimations and risk assessment planning [201].

Extended use of mobile phones can be helpful for plant pathologists and agronomists that use pictures of diseased plants taken by growers for data collection. The mass collection of images of symptomatic plants is processed by artificial intelligence (AI) capable of pattern recognition using deep learning algorithms that can attribute symptoms to specific plant pathogens [202]. This is further facilitated by open AI platforms (e.g., Google TensorFlow) that allows deep learning and big data processing to be accessible to researchers who can validate their algorithms without the need to implement supercomputing in their workplaces [203,204]. Eventually, any grower would be able to perform a diagnostic on a possible pathogen in the crop using his/her mobile phone for capturing images that are processed through mobile applications connected to AI databases. Collaboration between growers and researchers mediated by mobile apps and web platforms allows the processing of images of diseased crops combined with other data collected in the field (location, climate, etc.). These datasets would be analyzed for disease forecasting with minimal investment, thus facilitating decision making on crop management and/or taking preventive actions.

Box 1. Keys for the future.

Among the multidisciplinary strategies to face the challenges of sustainable vegetable cultivation in the Mediterranean Arc of Spain and areas of similar characteristics, we propose:

- acceleration of breeding programs, which can be facilitated by the new editing techniques available, including high-throughput phenotyping technologies
- resistance breeding at high temperature conditions (>30 °C)
- improvement of physical barriers to control virus vectors
- improvement of climate control in greenhouses
- increasing the research on epidemiology and plant virus evolution mechanisms and effects of mixed infections, in order to forecast resistance durability
- investigating the effects of CO₂ and temperatures on vector biology
- development of fast, reliable and cheap in situ diagnostics tools
- implementation of effective detection of emergences in greenhouses, nurseries, seed companies, etc. and reliable quarantine establishment
- identification of weeds as reservoirs of viruses and investigation of dispersion by pollinators
- alternatives to virus management, (e.g., developing RNAi-mediated control driven by nanocomposites)
- easy access for producers to information on pests and diseases (e.g., mobile applications)
- real-time communication of plant health authorities with producers/agronomists
- big-data analysis using AI platforms for decision making

7. Conclusions

In the last 30 years, novel viruses have appeared in the southeast of Spain at a rate of 0.9 viruses/year. Following this tendency, between 5 and 10 new virus species are expected to appear in the coming 10 years. It is hard to predict if this rate will increase in a scenario of climate change and increased trade in goods. However, although we can anticipate the arrival of novel viruses, the economic impact of the epidemics in the area can be minimized by improvements in different aspects of cultivation and technological developments. Here, we outline several technologies (Box 1), some of them still at an early stage, that will help to cope with current and novel virus epidemics affecting intensive horticultural crops in Spain and areas of similar characteristics.

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