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# Fighting Sharka in Peach: Current Limitations and Future Perspectives

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MC: drafted the manuscript and critically revised it;

FG, RS: critically revised the manuscript;

AB, VB, LC, BC, SD, LD, VD, SF, VI, AL, BM, AM, MP, TP, ThP, VS, and IV: critically revised the manuscript for important contents;

DB: conceived the manuscript and critically revised it;

### *Keywords*

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### *Abstract*

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Sharka, caused by Plum Pox Virus (PPV), is by far the most important infectious disease of peach [*P. persica* (L.) Batsch] and other *Prunus* species. The progressive spread of the virus in many important growing areas throughout Europe poses serious issues to the economic sustainability of stone fruit crops, peach in particular. The adoption of internationally agreed-upon rules for diagnostic tests, strain-specific monitoring schemes and spatial-temporal modeling of virus spread, are all essential for a more effective sharka containment. The EU regulations on nursery activity should be modified based on the zone delimitation of PPV presence, limiting open-field production of propagation materials only to virus-free areas. Increasing the efficiency of preventive measures should be augmented by the short-term development of resistant cultivars. Putative sources of resistance/tolerance have been recently identified in peach germplasm, although the majority of novel resistant sources to PPV-M have been found in almond. However, the complexity of introgression from related-species imposes the search for alternative strategies. The use of genetic engineering, particularly RNAi-based approaches, appears as one of the most promising perspectives to introduce a durable resistance to PPV in peach germplasm, notwithstanding the well-known difficulties of *in vitro* plant regeneration in this species. In this regard, rootstock transformation to induce RNAi-mediated systemic resistance to PPV would avoid the transformation of numerous commercial cultivars, and may alleviate consumer resistance to the use of GM plants.

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# Fighting Sharka in Peach: Current Limitations and Future Perspectives

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## Abstract

Sharka, caused by *Plum Pox Virus* (PPV), is by far the most important infectious disease of peach [*P. persica* (L.) Batsch] and other *Prunus* species. The progressive spread of the virus in many important growing areas throughout Europe poses serious issues to the economic sustainability of stone fruit crops, peach in particular. The adoption of internationally agreed-upon rules for diagnostic tests, strain-specific monitoring schemes and spatial-temporal modeling of virus spread, are all essential for a more effective sharka containment. The EU regulations on nursery activity should be modified based on the zone delimitation of PPV presence, limiting open-field production of propagation materials only to virus-free areas. Increasing the efficiency of preventive measures should be augmented by the short-term development of resistant cultivars. Putative sources of resistance/tolerance have been recently identified in peach germplasm, although the majority of novel resistant sources to PPV-M have been found in almond. However, the complexity of introgression from related-species imposes the search for alternative strategies. The use of genetic engineering, particularly RNAi-based approaches, appears as one of the most promising perspectives to introduce a durable resistance to PPV in peach germplasm, notwithstanding the well-known difficulties of *in vitro* plant regeneration in this species. In this regard, rootstock transformation to induce RNAi-mediated systemic resistance would avoid the transformation of numerous commercial cultivars, and may alleviate consumer resistance to the use of GM plants.

## Keywords

genetic engineering, fruit breeding, PPV virus, *Prunus persica* (L.) Batsch, RNAi.

## 2 **Introduction**

3 Sharka, caused by *Plum Pox Virus* (PPV), is the most devastating viral disease of peach and other  
4 stone fruits, resulting in significant economic losses (Cambra et al., 2006; Sochor et al., 2012). The  
5 progressive worldwide spread of this destructive disease requires a coordinated, focused effort to  
6 implement effective approaches to control its diffusion. Decades of research experience have made  
7 clear the need for transnational coordination involving not only researchers but all who are  
8 associated with the fruit industry including the phytosanitary services other than the growers  
9 themselves. An International Workshop on sharka disease in peach (Milan and Cesena, Italy, 2016,  
10 <https://sites.google.com/site/ppvsymposium2016>), gave the opportunity to review and update the  
11 state-of-art with particular attention to the main limitations and short-term perspectives.

12 The aim of this article is to provide a comprehensive picture of the current available  
13 possibilities to contrast sharka disease and to implement the resistance in peach, focusing on crucial  
14 aspects: the virus spread and management strategies within the EU regulatory framework; old and  
15 novel sources of genetic resistance (including the peach-related species almond and *P. davidiana*)  
16 and breeding perspectives; the application of novel genomics tools for the introgression of  
17 resistance and novel approaches for developing non-host resistance; the opportunity of using  
18 genetic engineering techniques, as already experienced in other fruit-tree species.

## 20 **Spread of sharka disease**

21 Currently, PPV is spreading in many countries, with the occurrence of several strains with  
22 different epidemiology and specific infective capabilities. However, precise information and  
23 tracking of PPV distribution, correlated with the specific strain(s), is lacking in many peach  
24 growing regions. Little is known about the epidemiology of the Eastern Europe strains. Five new  
25 strains have been discovered in the last 10 years. The general framework of the PPV strains  
26 currently established includes, in addition to the most common PPV-M, -D and -REC, the strains -T  
27 (Turkey, Albania), -An (Albania), -EA (Egypt), -W (Canada, Ukraine, Russia, Latvia), -CR  
28 (Russia) and -C (Moldavia, Belarus and Russia), the last two generally limited to cherry.  
29 Homologous recombination plays an important role in PPV evolution (Garcia et al., 2014). Several  
30 features including higher aphid transmission rates, a reduced latency period, faster virus diffusion in  
31 the orchards from primary infected plants and broad host range, confer to PPV-M the highest  
32 epidemicity in peach (Dallot et al., 2003). Information about the dynamics of host infection as it  
33 affects disease spread remains scarce.

34 Several strategies have been deployed to manage sharka disease, depending on the epidemic  
35 context. Eradication, based on orchard surveillance and removal of infected plants has been widely  
36 adopted in Western Europe and North America, whereas tolerant cultivars have been used in

37 Eastern Europe (particularly for plum). In the former case, it becomes imperative to organize and  
38 carry out efficient strain-specific monitoring schemes implementing spatial-temporal modeling of  
39 disease spread. In France, surveillance intensity varies according to disease risk and control  
40 activities of the Plant Health Services are supported by skilled private professional organizations.  
41 The heterogeneity of PPV diagnostic tests is one of the main limitations for disease management.  
42 The test method(s) should be established by specific internationally agreed-upon rules. More rapid  
43 and accurate techniques for PPV diagnosis on candidate plants entering into the certification system  
44 are required, along with the ability to detect virus infection during the latency period. Novel  
45 techniques, such as Digital Droplet PCR (ddPCR) (Gutiérrez-Aguirre et al., 2015) with the potential  
46 to detect up to one copy of viral RNA and isothermal amplification by reverse transcription-  
47 recombinase polymerase (Zhang et al., 2014), applicable directly on-field, are quite promising. To  
48 develop the next generation of novel diagnostics, close cooperation among all the interested actors,  
49 including entomologists will be important. For more information on PPV management strategies,  
50 see Rimbaud et al., (2015).

#### 51 **Regulatory framework**

52 Considering the spread of sharka in Europe, the disease can no longer be controlled through  
53 quarantine legislation only, but the latter must be combined with the regulation of the Plant  
54 Propagating Material Quality. Evidence of such changes in the EU policies are found within  
55 documents in preparation which are now discussed in the context of the Harmonization of the  
56 Certification Scheme for Fruit Plants, particularly the Annex to the Commission Implementing  
57 Directive amending Annex IV Part A Section II of Directive 2000/29/EC on protective measures  
58 against the introduction in the Community of organisms harmful to plants or plant products and  
59 against their spread within the Community. Concerning PPV, many of the directives contained in  
60 the documents are not compatible with the epidemiology of the disease and its danger. For example,  
61 the time intervals provided for diagnostic tests are too long for an effective management. Another  
62 controversial and unexplained aspect is the criterion allowing the possibility to maintain mother  
63 plants under field condition in endemic areas.

64 The control of nursery plant material and trading pathways is critical. Insufficient controls  
65 on hundreds of new peach varieties offered to nurseries and growers every year, and the sporadic  
66 presence of PPV infected material in nurseries, along with the trade and/or exchange of  
67 uncontrolled materials for grafting by fruit growers, set up ideal conditions for national and  
68 international virus spread. Moreover, the release of the European Plant Passport according to the  
69 EU regulations and upon Phytosanitary Service control does not guarantee preventative testing of  
70 trade materials. In this regard the inspection and control on breeding and nursery materials, the  
71 utilization of virus-free (VF) certified plants, the restriction of nursery activities to pest-free areas

72 or under screenhouse, and the safeguarding of pest-free areas are all imperative. For the  
73 management of sharka disease it is essential that:

74 i) the effective and adequate application of a mandatory certification system for propagation  
75 material be used for all EU members;

76 ii) the zone delimitation of European fruit growing areas based on PPV presence should  
77 limit the nursery production in open field only in virus-free areas (alternatively, in secure  
78 screenhouses);

79 iii) PPV isolates detected in new foci be characterized molecularly and serologically for risk  
80 assessment evaluation;

81 iv) PPV infection status of any new cultivar released and its behavior toward PPV infection  
82 be evaluated at least for the most common PPV strains (M, D and Rec).

83 In conclusion, EU legislation must consider all these aspects since PPV should be regarded  
84 as a quarantine pest and the level of alert be enforced and not reduced.

85

#### 86 **Source of resistance to sharka and breeding perspectives**

87 The results of two decades of research confirm the absence of immune or resistant cultivars  
88 in peach germplasm, although a general low susceptibility to PPV-D strain has been reported  
89 (Rubio et al., 2012). The introgression of resistance from peach-related species, such as *Prunus*  
90  *davidiana* (Carrière) (Pascal et al., 1998) and *Prunus dulcis* (Webb) (Pascal et al., 2002; Martínez-  
91 Gómez et al., 2004), has been unsuccessful so far, mainly due to the difficulty in combining PPV  
92 resistance with traits suitable for the peach marketing (Moing et al., 2003). Most of the limitations  
93 come from the low rates of resistant individuals produced by crossbreeding and by the many  
94 unfavorable traits expressed by F<sub>1</sub> hybrids, requiring at least several rounds of backcrossing to  
95 recover ‘commercial’ fruit traits. The absence of molecular markers associated with resistance,  
96 which would facilitate the selection of resistant seedlings, adds to the complexity of breeding PPV  
97 resistant cultivars. The introduction of resistance from *P. davidiana* has been recently suspended by  
98 French breeding programs in favor of the introgression from almond. As a result of the  
99 collaboration between INRA-Avignon (France) and CEBAS-CSIC (Spain) several almond cultivars  
100 resistant to PPV-M were identified, including ‘Del Cid’, and have been chosen by INRA as resistant  
101 parents for building hybrid populations. Nevertheless, the experience within the Italian PPVCON  
102 project suggests that the use of almond as a resistance donor could suffer from the same limitations  
103 observed in resistant peach selections coming from UCD hybrids [(‘Padre’(almond) x ‘54P455’  
104 (peach)) x ‘Hesse’ (peach) x self], characterized by poor fruit quality (Liverani et al., 2011).



105           Among other approaches attempted to confer sharka resistance, the use of aphid-resistant  
106 peach selections proved to be ineffective, since the trait was unable to ensure a protection against  
107 virus transmission in endemic areas (Liverani et al., 2015).

108           The extensive evaluation of more than 300 peach cultivars from core collections and  
109 recently released varieties showed the presence of a small number of accessions highly tolerant to  
110 the PPV-M strain (e.g. infectable by the virus but asymptomatic or developing only mild symptoms,  
111 particularly on fruit) (Liverani et al., 2011). A promising resistant selection (e.g. ‘Spasena’) derived  
112 from the resistant parent ‘Dupnsika’ (Gabova et al., 1994) is currently under evaluation at the Fruit  
113 Growing Institute of Plovdiv (Bulgaria). Notably, the selection process occurring in open-field  
114 conditions within endemic sites, allows an increased reliability of resistance evaluation results.  
115 Indeed, as observed from field trials in Italian endemic areas, PPV-M was able to infect in just a  
116 few years about 70% of advanced selections, found resistant after several years of greenhouse  
117 ‘heavy test’ evaluations, i.e. grafting on already infected GF305 (Liverani et al., 2015). In  
118 perspective, the outdoor trials in endemic areas appear as a more practical, cost-effective and  
119 reliable solution for the screening of promising selections.

120           An interesting research field is the use of resistant rootstocks, such as the almond cultivar  
121 ‘Garrigues’, to induce resistance in the scion (Rubio et al., 2013). The postulated mechanism  
122 responsible for the prevention or recovery from infection is the systemic transmission by graft  
123 (almond) to scion (peach) of a silencing signal. Apart from the repeatability of the experiment in  
124 peach cultivars other than the already tested ‘GF305’, some other questions are raised about its  
125 durability in time and in the field. Most important, considering the general tolerance of peach  
126 species to PPV-D strain, further studies should ascertain the stability of this mechanism against the  
127 most virulent PPV-M strain.

128           In conclusion, a divergent strategy for incoming breeding programs appears evident among  
129 some Italian and French research groups: the former is aiming at (short-term) development of  
130 cultivars highly tolerant to PPV-M (no symptoms, at least on fruit) cultivars suitable for the  
131 preservation of a peach industry in endemic areas; the latter attempts to introduce durable resistance  
132 (no virus replication in the tree) from almond (as PPV-M resistant parent or as a resistance-inducing  
133 rootstock). The introduction of tolerant plants is a matter of debate, since epidemiologists are  
134 concerned about the possible development of more aggressive PPV strains raising by recombination  
135 from mixed PPV infections and also for increasing the difficulties of implementing containment  
136 strategies on asymptomatic plants, since a PPV inoculum reservoir still persists in the area.

137

138 **Molecular and functional genomics**

139 As demonstrated by several studies, the resistance to sharka conferred by the *P. davidiana*  
140 clone 'P1908' is of a quantitative nature, regulated by several QTLs with small effects, often  
141 variable across years (Decroocq et al., 2005). Linkage mapping experiments performed on  
142 progenies derived from different cross combinations of 'P1908' and/or SD hybrids ('P1908' x  
143 'Summergrand') also demonstrated that QTL numbers and positions are affected by the genetic  
144 background of the peach parents (Marandel et al., 2009; Rubio et al., 2010). Currently, candidate  
145 gene(s) or molecular markers associated with PPV resistance have not been identified in *P.*  
146 *davidiana* or derived peach hybrids, hindering the short-term launching of marker-assisted selection  
147 (MAS) programs. The availability of the Peach Genome reference sequence and a 9K SNP array  
148 platform (Verde et al., 2012), in addition to the availability of novel, powerful 'omics' tools may  
149 accelerate the identification of the genetic basis of PPV resistance in peach related species. For  
150 example, a Genotyping-By-Sequencing approach has been recently adopted to develop high-density  
151 genome markers for the introgression of resistance from almond (French FruitSELGEN project).  
152 While this approach is undoubtedly promising at the scientific level, concerns still persist about the  
153 real possibility of developing valuable cultivars from peach-almond hybrid(s) in the short-medium  
154 term.

155 The main bottlenecks for the identification of the genetic bases of PPV resistance, are still  
156 the cost, complexity and time-consuming phenotyping procedures, given the well-known  
157 unresolved issues:

158 i) The lack of standardized phenotyping methods among research groups, i.e 'heavy test'  
159 (see above) vs. 'standard test' (by inoculating buds from infected plants onto the accessions to be  
160 challenged), restricting the cross-validation of results.

161 ii) A subjective and non-uniform interpretation of visual symptoms and discrepancies in  
162 terminology, particularly for 'resistance' and 'tolerance'. An alternative scoring system to  
163 overcome the strong subjectivity of symptoms evaluation is still lacking. A proposal for a  
164 standardized terminology is shown in Table 1.

165 iii) The long-term requirement of evaluation trials for a reliable assessment of resistance. No  
166 quick protocol for resistance evaluation has been implemented so far and is not recommended, since  
167 the alternance of resting and growing periods is obviously important for the reliability of the trial.

168 iv) The reliability of 'in-field' resistance evaluation vs. 'screenhouse' testing.

169 Knowledge of the mechanism of PPV-peach interaction at physiological, proteomic,  
170 metabolic and gene expression level are still scarce, although it may have practical implication for  
171 disease management (reviewed by Clemente-Moreno et al., 2015) or for the development of  
172 biomarkers to detect early PPV infection, especially for nursery mother trees. Recently, proteome  
173 analysis of PPV-infected peach plant showed that infection affected the abundance of proteins

174 related to photosynthesis, carbohydrate and amino acid metabolism (Clemente-Moreno et al., 2013).  
175 RNA-seq of peach leaf transcriptome after PPV-D infection demonstrated the complexity of plant  
176 response and the critical role of early responsive genes as a reaction against virus replication and  
177 translocation, before symptoms development (Rubio et al., 2015). Unfortunately, the experiments  
178 above were not performed on PPV resistant accessions, thus there are not information about  
179 candidate genes involved in sharka resistance in peach so far.

180 Developing non-host resistance in peach is a new frontier of research. Viruses encode a  
181 limited number of essential proteins, since recruit plant proteins (host factors) for every stage of the  
182 infection cycle (Garcia et al., 2015; van Schie and Takken, 2014). Gene(s) coding for such factors  
183 can be considered as susceptibility (S) genes and their mutation or loss of function limit the ability  
184 of the pathogen to cause disease. For example, recessive resistance to some *Potyvirus*es, PPV  
185 included, is associated with mutations of genes belonging to the eukaryotic translation initiation  
186 factors (*eIF*) family in several plant species, thus representing an excellent candidate to also confer  
187 non-host resistance in peach. Different tools have been deployed to explore the possibility of  
188 developing a non-host resistance in peach:

189 i) EcoTILLING approach, searching for peach variants in which susceptibility gene(s) are  
190 deleted or non-functional. Nevertheless, as a consequence of reduced genetic diversity, peach shows  
191 the lowest number of variants in comparison with other *Prunus* species. All promising individuals  
192 are hybrids (peach crossed to almond or peach wild related species);

193 ii) TILLING approach, by creating *de novo* non-functional host genes through chemical  
194 mutagenesis (EMS). Three thousand individuals derived from the susceptible peach rootstock  
195 ‘GF305’ are currently under screening by whole-genome re-sequencing to evaluate the mutation  
196 rate after EMS treatment and the mutation rate in susceptibility genes.

197 iii) S gene(s) silencing and/or Genome Editing (discussed in the next section).

198 The application of novel ‘omics’ approaches holds great promise for unraveling the  
199 complexity of the genetic mechanisms regulating sharka resistance. This knowledge is essential for  
200 developing useful molecular tools in breeding PPV resistant varieties as well for implementing  
201 more efficient disease management strategies. The development of non-host resistance in peach is  
202 an ambitious target, that is now moving its first steps.

203

## 204 **Introducing resistance in peach by genetic engineering**

205 In light of the complexity of breeding PPV-resistant peach cultivars by conventional  
206 strategies, as determined by the absence of resistant cultivars, by the low genetic diversity in peach  
207 germplasm, and by the ineffectiveness of introgression from related species, genetic engineering

208 (GE) approaches appear an alternative strategy at the current status of knowledge (Ilardi and  
209 Tavazza, 2015).

210 Long-term evaluations, lasting over 20 years, in both greenhouse and endemic areas  
211 throughout Eastern Europe, have demonstrated the effectiveness of GE approaches to confer stable  
212 and durable sharka resistance in plum (Scorza et al., 2016). PPV resistance in transgenic  
213 ‘HoneySweet’ plum is based on RNA interference (RNAi), triggered by a complex multi copy  
214 insertion of an inverted repeat/hairpin configuration of the PPV coat protein (PPV-CP) transgene,  
215 activating the production of 25-26nt class siRNA and ultimately leading to viral RNA degradation  
216 (Kundu et al., 2008). A more detailed knowledge of PTGS mechanisms has led to the development  
217 of more effective tools for RNAi-mediated engineered resistance. In particular, intron hairpin RNA  
218 (ihpRNA) constructs have proven to be highly effective inducers of local and systemic resistance  
219 against PPV in both model species *N. benthamiana* (Pandolfini et al., 2003; Di Nicola-Negri et al.,  
220 2005) and *P. domestica* (Hily et al., 2007; Monticelli et al., 2012). In addition, PPV-derived  
221 ihpRNA constructs were able to induce high resistance to a wide range of PPV strains (Di Nicola-  
222 Negri et al., 2010; Ravelonandro et al., 2014) also under variable abiotic and biotic conditions that  
223 are known to have a negative impact on gene silencing in plants (Di Nicola et al., 2014).  
224 Advantages of ihpRNA constructs include the high flexibility (it is possible to target multiple  
225 sequences from different PPV genomic regions or different PPV strains, Wang et al., 2013), high  
226 stability of siRNAs production, no documented interference with the endogenous RNA silencing  
227 machinery, robust and durable antiviral resistance and absence of transgene-derived proteins. Some  
228 questions related to undesirable consequences of RNAi still remain, such as off-target effects  
229 leading to changes in the host transcriptome; trade-off between defense and growth/development  
230 processes; and virus escape from silencing (Fuentes et al., 2016). However, the risk of such off-  
231 target effects can be identified by a well-developed science based risk assessment and reduced by a  
232 continuous monitoring as defined in a proper post-monitoring program to be applied after the  
233 release of the new events.

234 However, the application of genetic transformation techniques in peach has been limited by  
235 the difficulties in developing efficient regeneration and transformation protocols. Nevertheless, a  
236 protocol for *in vitro* regeneration via organogenesis, has been already developed for one of the most  
237 widely used peach rootstocks ‘GF677’ (Sabbadini et al., 2015). The protocol may be further  
238 improved by using different selection markers since ‘GF677’ appears to be naturally rather resistant  
239 to the antibiotic kanamycin. At present, the availability and the already demonstrated capabilities of  
240 many viral-derived constructs to induce RNAi against PPV, jointly with a high probability of  
241 transforming ‘GF677’, make the hypothesis of rootstock engineering one of the most feasible and  
242 promising for peach cultivars (MIUR - PRIN VIREs project). Root-to-scion (and scion-to-root)

243 siRNAs transfer has been already demonstrated in several annual model plants (reviewed in Pyott  
244 and Molnar, 2015). Rootstock transformation would be appealing, for several reasons: the scion  
245 would maintain its genetic background; no gene flow, because pollen and seeds would not be  
246 allowed to be produced by the genetically modified rootstock; the same transgenic rootstock can be  
247 used for many cultivars, avoiding the transformation of each single accession; and it may simplify  
248 many aspects related to the opinion of the consumers on GM plants (Lemgo et al., 2013). Recently,  
249 this approach has been successfully applied in a cherry rootstock to induce RNAi-mediated  
250 systemic resistance to Prunus Necrotic Ringspot Virus (Song et al., 2013; Zhao and Song, 2014),  
251 although the stability and durability of this approach requires further evaluation. On the contrary,  
252 transmission of RNA silencing was not observed in non-transgenic scions in apple (Flachowsky et  
253 al., 2012), and thus, additional research is required to set-up the appropriate strategies for an  
254 efficient silencing through grafting (Gohlke and Mosher, 2015).

255 While the ability of PPV-resistant ‘HoneySweet’ plum to transfer the silencing signal has  
256 not been demonstrated, the idea to use it directly as a rootstock in European peach growing areas is  
257 attractive. The main limitation arises from the implementation of experimental field trials, which  
258 require the approval of competent authorities. ‘HoneySweet’ and other reported GE and  
259 conventional PPV resistant *Prunus* should be field tested as rootstocks (and as scions) in PPV  
260 endemic areas. This work requires that competent authorities evaluate GE field tests on scientific  
261 merit and realistic biosafety issues, and that local authorities guarantee the safety of these plantings  
262 against destruction by radical GE opponents.

263 In spite of the success obtained through GE approaches to induce virus resistance in several  
264 crops, public concerns over the potential ecological impact of GE organisms and/or products  
265 strongly limit their use in Europe. In this sense, the approval of ‘HoneySweet’ by US authorities is a  
266 paradigm shift for fruit trees in terms of the application of GE technology (Scorza et al., 2013). Risk  
267 assessment is an integral part of GE plant production, and the time and costs that it requires should  
268 be added to those of plant transformation, selection and evaluation. In addition to the molecular  
269 analysis of plants, the biochemical characterization of fruits and the evaluation of resistance, further  
270 information may be required from authorities and consumers, including plant-virus and plant-insect  
271 interactions, gene flow and the potential risk of off-target gene silencing (All of which were  
272 addressed for ‘HoneySweet’ in the U.S. regulatory dossiers). Excluding marker proteins, the risks  
273 associated with newly expressed proteins (i.e. allergenicity or toxicity) would not be meaningful for  
274 RNAi-based engineering, due to the lack of viral proteins produced by RNAi plants.

275 Genome editing is a novel and promising type of GE, based on the use of engineered  
276 nucleases for the introduction of mutations at target genomic sequences. Despite the growing  
277 positive opinion about its application from authorities and consumers, it is still hardly applicable in

278 peach. In order to maintain clonal stability in vegetative propagated plants, the application of  
279 genome editing techniques requires the development of effective protocols of plant regeneration  
280 from protoplast culture, unavailable in peach. Lacking the possibility of transient expression, stable  
281 transformation with the Cas9 system will be necessary (Jia and Wang, 2014; Xu, 2013), which will  
282 then require a round of backcrossing or self-pollination for its removal after editing procedures and  
283 regeneration. The mandatory germline transition generates a novel cultivar that must then be  
284 evaluated before market introduction. In addition, some concerns still persist about how this  
285 procedure of developing non transgenic plants using GE techniques would be classified by the  
286 authorities. Despite this, the resistance conferred by the editing of susceptibility genes (S) to confer  
287 PPV resistance is an interesting perspective. The *eIF4E(s)*, *cPGK*, *DBP1* and/or *PpDDXL* genes  
288 recently functionally characterized in peach represent good candidates for the application of  
289 Genome Editing (Castelló et al., 2011; Poque et al., 2015; Huang et al., 2010). However, because S  
290 genes have a function other than as a compatibility factor for the pathogen, the side effects caused  
291 by their mutation demand a one-by-one assessment of their usefulness for application. Plant  
292 regeneration from protoplast cell lines has never been demonstrated in peach and this remains the  
293 major issue for the application of genome editing in heterozygous vegetatively propagated plants.  
294 Therefore, current knowledge of RNAi approaches seem more suitable to yield positive results.

295 A relatively recent technology for rapid cycle breeding ('FastTrack') developed in other  
296 species (apple, plum, etc.) is a breeding system that uses a GE tree expressing a flowering pathway  
297 gene, such as *FT* gene orthologs, to obtain fruiting trees in one year (or even less) (Flachowsky et  
298 al, 2011; Srinivasan et al, 2012). Shortening the juvenile stage in peach would accelerate the  
299 conventional breeding procedures for the introgression of sharka resistance from peach-related  
300 species. Also, the final resulting trees would not be GE unless the PPV resistance gene used is a  
301 transgene.

302

### 303 **Conclusions**

The containment of sharka disease spread is one of the most important priorities of the European peach industry. The complexity of this phytosanitary issue does not allow simple, rapid solutions. The application of preventive measure with the maximum alert level and, possibly, their reinforcement through the implementation of more effective management strategies, are of utmost importance. The adoption of internationally agreed-upon rules for diagnostic tests, strain-specific monitoring schemes and spatial-temporal modeling of virus spread, are all essential for a more effective sharka containment. The EU regulations on nursery activity should be modified based on the zone delimitation of PPV presence, limiting open-field production of propagation materials only to virus-free areas. Prevention should be combined with the introduction of genetic resistance in a

reasonable time, but not at the expense of high fruit quality productions. A part from still to be verified existence of intraspecific sources of PPV resistance in peach, the complexity of introgression from related-species imposes the search for alternative strategies. Currently, the use of genetic engineering, particularly RNAi-based approaches, appears as one of the most promising perspectives, notwithstanding the well-known difficulties of *in vitro* plant regeneration in this species. Rootstock transformation to induce RNAi-mediated systemic resistance to PPV would avoid the transformation of numerous commercial cultivars, and may alleviate consumer concerns to the use of GM plants. In this regard, the use of genetic engineering approaches represent a fundamental opportunity, and as such it should be supported not only from a technical-scientific view point, but also in a broader socio-political context.

304

### 305 **Conflict of Interest**

306 The authors declare no conflict of interest.

307

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314

### 315 **Author contributions**

MC: drafted the manuscript and critically revised it;

FG, RS: critically revised the manuscript;

316 AB, VB, LC, BC, SD, LD, VD, SF, VI, AL, BM, AM, MP, TP, ThP, VS, and IV: critically revised  
317 the manuscript for important contents;

318 DB: conceived the manuscript and critically revised it;

319 All authors: read and approved the final manuscript

320

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471  
 472 Table 1. Proposal for a standardized terminology. \*Immunity: non-host resistance (plant cannot be  
 473 infected); Resistant: negative ELISA and RT-PCR assay, no visible symptoms; Susceptible:  
 474 positive or negative ELISA, positive RT-PCR assay, symptoms on plant organs from absence  
 475 (tolerant) to severe (sensitive) (mandatory specification of organ/tissue). ‘Recovery reaction’,  
 476 characterized by an initial low infection (RT-PCR positive only) later followed by no detectable  
 477 infection (RT-PCR negative).

478

Classification	Visual Symphoms	ELISA	RT-PCR
Non-host			
Immune	-	-	-
Host			
Resistant	absent	-	-
Susceptible	absent (tolerant) to severe (sensitive)	+/-	+
Recovery	absent	-	+ to -

479