

1

2 **Detection of an unstable minor QTL linked to fire blight 3 resistance on linkage group 16 of *Malus fusca***

4 **Ofere Francis Emeriewen^{1*}, Klaus Richter², Annette Wensing³, Mickael Malnoy⁴ and
5 Andreas Peil^{1*}**

6
7 ¹Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Breeding
8 Research on Fruit Crops, Dresden, Germany

9 ²Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance
10 Research and Stress Tolerance, Quedlinburg, Germany

11 ³Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Plant
12 Protection in Fruit Crops and Viticulture, Dossenheim, Germany

13 ⁴Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all 'Adige, Italy

14
15 *Correspondence: ofere.emeriewen@julius-kuehn.de; andreas.peil@julius-kuehn.de

19 **Abstract**

20 **Objective**

21 *Erwinia amylovora* causes fire blight disease in *Malus*. A strong resistance QTL (*Mfu10*) was
22 previously detected on linkage group 10 of *Malus fusca* accession MAL0045, using several
23 strains of the bacterium. As no strain capable of breaking the resistance of MAL0045 has been
24 found, it was hypothesized that a second resistance factor contributes to the fire blight resistance
25 of MAL0045. However, to date, no minor locus has been detected with previously published
26 strains of the bacterium. We detected a minor QTL only on a subset of a population following
27 inoculation with strain Ea1038, which heterologously expresses an effector in a derivative of
28 isolate Ea3049. Two genetic maps of MAL0045, one scarce, the other dense with markers, were
29 used for QTL analyses.

30 **Results**

31 *Mfu10* was detected on LG10 with Ea1038, as was previously with Ea3049. Although no other
32 QTLs of significant LOD was previously detected in other linkage groups with Ea3049, a QTL
33 of significant LOD was detected on LG16 (*Mfu16*) after inoculation of a subset of 76
34 individuals with Ea1038, but only using the dense genetic map. *Mfu16* improved the effect of
35 *Mfu10*. However, when the number of individuals inoculated with Ea1038 was increased to
36 121, *Mfu16* was no longer detected in the dense genetic map. We hypothesize some factors,
37 which might be responsible for the instability of this QTL.

38 **Keywords:** *Erwinia amylovora* strains, MAL0045, *Mfu10*, *Mfu16*, *Malus fusca* fire blight
39 resistance loci

40

41

42 **Introduction**

43 The bacterium, *Erwinia amylovora*, causes fire blight – a devastating disease of the
44 domesticated apple (*Malus domestica* Borkh.) and related species (*Malus* spp.) [1-4]. The
45 mechanisms by which the pathogen invades and causes disease in susceptible hosts have been
46 extensively reviewed [2, 5]. Similarly, the molecular strategies employed by resistant hosts for
47 the recognition of *E. amylovora* elicitors are well documented [2, 4, 6]. *Malus* host resistance
48 is mostly quantitative, evidenced by the segregation of resistant and susceptible phenotypes in
49 *Malus* populations [7-11]. Quantitative trait loci (QTLs) for fire blight resistance have been
50 detected in apple cultivars and wild apple species accessions [reviewed in 4]. Although most
51 apple cultivars are more susceptible to the disease, their QTLs are faster to introgress but not
52 sufficient to provide strong resistance as those of their wild relatives [8-11], for which fire blight
53 resistance candidate genes have been proposed [12-15], and in one instance, functionally proven
54 [16].

55 A strong fire blight resistance QTL (*Mfu10*) was detected on linkage group (LG) 10 of the wild
56 apple accession, *Malus fusca* MAL0045 [9]. The stability of *Mfu10* has been demonstrated
57 using several strains of *E. amylovora* differing in virulence/aggressiveness [17-19]. In
58 particular, the highly aggressive Canadian strain, Ea3049, and the mutant strain ZYRKD3-1,
59 both of which break down the resistance locus of another wild *Malus* genotype, *Malus* ×*robusta*
60 5 (Mr5) [20, 21], could not breakdown *Mfu10* [17, 18]. However, unlike in Mr5, where minor
61 QTLs were detected following inoculation with Ea3049 [22], no minor QTLs were detected in
62 MAL0045 following inoculations with four different strains [19].

63 **Rationale and methods**

64 It was previously reported that a switch from cysteine amino acid (C-allele) to serine amino
65 acid (S-allele) in the *avrRpt2EA* effector protein sequence of *E. amylovora* at position 156 is
66 responsible for virulence and resistance breakdown in Mr5 [21] but also aggressiveness in other
67 *Malus* host [4]. Ea3049 possesses the S-allele contributing to the high virulence/aggressiveness
68 of this strain.

69 That MAL0045 itself is highly resistant to Ea3049 but the average PLL of the progeny increased
70 to 62.4 compared to of 22.6 after inoculation with strain Ea222 [17], led us to speculate that
71 there might be a second factor contributing to the resistance of MAL0045, and/or the SNP in
72 the *avrRpt2EA* effector of Ea3049 [21] might not be the only virulence factor of this strain. We
73 inoculated the original mapping population (05210 individuals) derived from MAL0045 ×
74 ‘Idared’ cross [9], with a mutant strain of Ea3049. In the meantime, we developed a dense
75 genetic map of MAL0045 using genotyping-by-sequencing generated SNPs incorporated with
76 microsatellite markers (SSRs), for MAL0045-derived progenies namely: 05210 and 09260
77 individuals [19].

78 The strain used in this study, Ea1038, is a derivative of Ea3049 with the chromosomal S-allele
79 of the *avrRpt2EA* effector deleted and complemented with the C-allele on an expression vector.
80 Artificial shoot inoculation was performed on scions of up to 10 replicates of each progeny
81 individual grafted onto rootstock M9, by cutting the youngest leaves with a pair of scissors
82 dipped into bacterial inoculum (10^9 cfu/ml). Disease necrosis was measured in centimeters 27

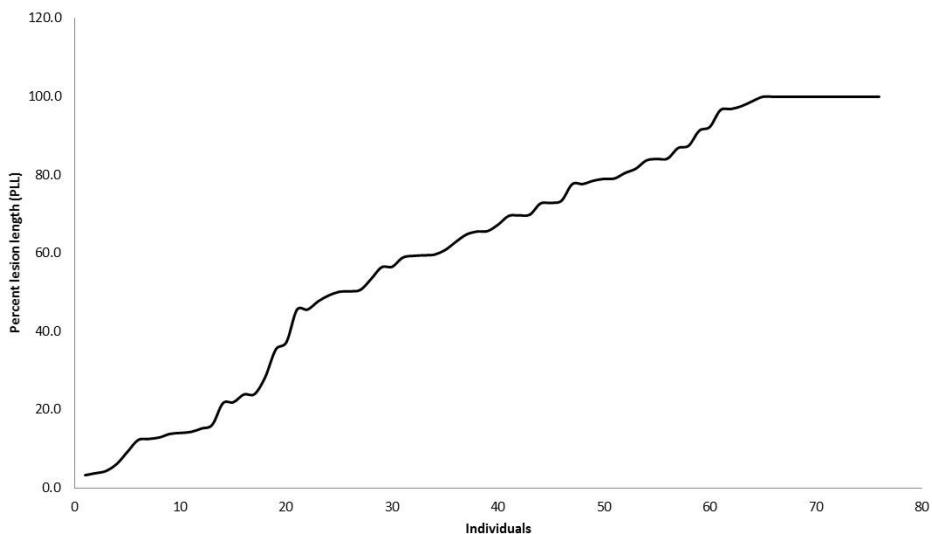
83 days post inoculation (dpi) and converted into percent lesion length (PLL) by dividing the
84 necrotic shoot by the total shoot length and multiplying by 100. The preliminary QTL mapping
85 was done with the phenotypic data of 76 inoculated plants. Subsequently, the number of
86 phenotyped individuals could be increased to 121 individuals. These data were used for a
87 second QTL mapping. The calculated average of PLL of all replicates of each individual was
88 used for Kruskal-Wallis analysis and interval mapping using MapQTL 5.0 [23]. The first
89 incomplete genetic map of MAL0045 [9, 24] and the recently developed dense map [19] served
90 as templates for QTL analysis. This map was established using 112 individuals of the 05210
91 population and 36 individuals of the 09260 population, 148 individuals in total.

92 SAS (SAS Institute) GLIMMIX (generalized linear mixed model) analysis was performed to
93 determine whether the effects of detected loci were significantly different. For this analysis,
94 phenotypic values (PLL) of each progeny individual as well as their marker alleles were
95 employed.

96 **Results**

97 Of 112 individuals of 05210 population used to develop the map [19], it was only possible to
98 phenotype 76 individuals by artificial shoot inoculation with Ea1038 in 2017. The distribution
99 of PLLs for all 76 individuals is shown in Figure 1. For these individuals, 61.1 % and 65.7 %
100 were the mean and median PLLs, respectively. Only five individuals recorded PLLs below 10.0
101 with 3.3 the lowest. Fifty-two individuals recorded PLLs over 50 with 100 recorded as the
102 highest for twelve individuals. For the parents, whilst a PLL of 9.6 was recorded for MAL0045,
103 100 % was recorded for 'Idared'.

104



105

106 **Figure 1.** Distribution of PLL of seventy-six individuals of 05210 population phenotyped with Ea1038

107

108

109

110 The averages of all replicates for each of the 76 individuals were used for Kruskal-Wallis
111 analysis and interval mapping with the newly developed map as template. Kruskal-Wallis
112 analysis showed that markers on LG10 (highest K value = 37.2), LG16 (highest K = 13.0) and
113 LG17 (highest K = 11.6) correlated with fire blight resistance (Table 1). The most significant
114 correlation with resistance was observed in LG10 where individuals inheriting the resistant
115 allele of the marker with the highest K value (Sca_304010_602250) possessed 43.8 % less
116 necrosis than individuals inheriting the susceptible allele (i.e. difference between mean ll and
117 mean lm genotypic segregation). For LG16 and LG17, the differences were 28.1 %
118 (Sca_304000_613165) and 22.1 % (CH05b06_L4), respectively (Table 1). With the first
119 incomplete map, only markers on LG10 showed correlation with fire blight resistance (data not
120 shown).

121 **Table 1.** Kruskal-Wallis analysis results for some markers on the three linkage groups of the dense
122 genetic map showing strong correlation with fire blight resistance following inoculation with Ea1038

Marker	LG	Position (cM)	K	PLLs of plants with	
				Susceptible alleles	Resistance alleles
CH03d11	LG10	38.127	35.6*****	85.1	42.2
Sca_313304_278642	LG10	39.404	36.2*****	84.9	41.8
FR481A	LG10	41.421	31.3*****	84.2	44.4
Sca_304010_602250	LG10	43.152	37.2*****	84.7	40.9
FR149B	LG10	48.740	27.8*****	81.7	44.7
<hr/>					
Sca_300922_5406043	LG16	12.568	11.6****	76.8	50.3
Sca_315074_14818	LG16	14.715	12.9****	76.8	49.1
Sca_304000_613165	LG16	16.650	13.0****	76.8	48.7
Sca_300922_6119061	LG16	16.013	12.8****	76.5	48.7
<hr/>					
Sca_315325_42556	LG17	0.000	11.2****	72.8	50.1
Sca_313414_17371	LG17	2.172	10.9****	73.2	51.2
Sca_307499_834166	LG17	2.204	10.9****	72.9	51.2
CH05b06_L4	LG17	2.808	11.6****	72.1	50.0

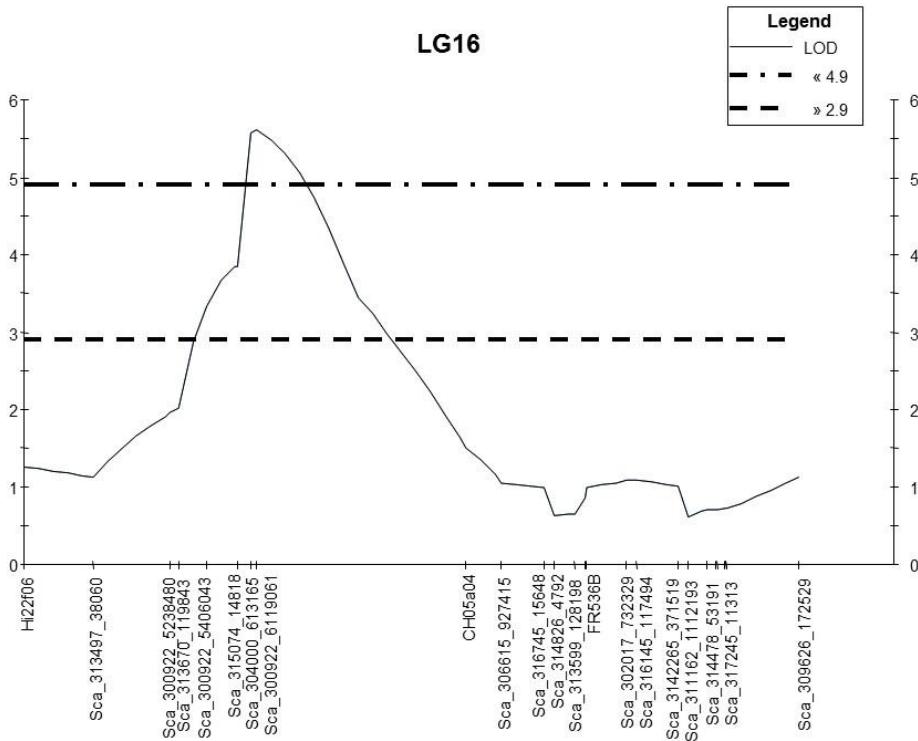
123 *K* Value of Kruskal-Wallis analysis (significance levels: **=0.05, ****=0.005, *****=0.0001); *PLL* percentage lesion length

124

125 Interval mapping with the genome wide (GW) threshold of 4.9 identified two QTLs of
126 significant LOD scores on LG10 and LG16. No significant QTL was found on LG17. The QTL
127 detected on LG10 is *Mfu10*, previously detected with other strains of *E. amylovora* since it is
128 located in the same interval between CH03d11 and FR149B with markers possessing highly
129 significant *K* values ($P = 0.0001$) (Table 1). However, the QTL on LG16 (*Mfu16*) is a novel
130 minor QTL (Figure 2) never previously detected with any strain. *Mfu16* was not detected in the
131 scarce map with Ea1038. Interval mapping results was in agreement with Kruskal-Wallis
132 analysis as SNP markers on LG16 with highly significant *K* values (Table 1) possessed the
133 highest LODs and appear underneath the QTL plot (Figure 2). The significance and interaction
134 between *Mfu10* and *Mfu16* were determined using SAS GLIMMIX analysis. Fire blight
135 resistance was significantly stronger when resistance alleles of both loci were present in
136 individuals compared to when individuals possessed only *Mfu10* or *Mfu16* resistance alleles
137 (Figure 3). Further, the resistance level of *Mfu10* alone was significantly stronger than the
138 resistance level of *Mfu16* alone.

139

140



141

142

143 **Figure 2.** LOD plot of interval mapping for the detected QTL on LG16 showing the significance at the
144 chromosome level threshold of 2.9 and genome wide threshold of 4.9, with the markers

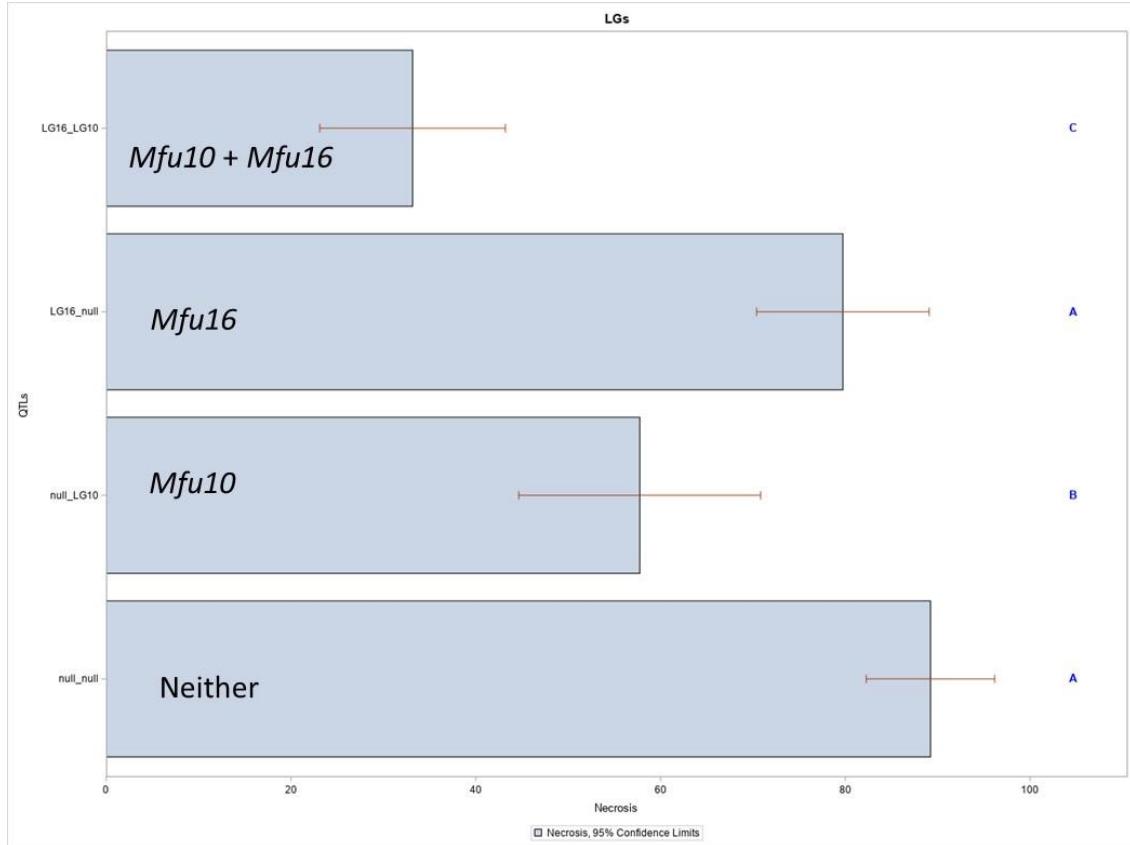
145

146

147 A total of 121 individuals could be inoculated with Ea1038 from 2017 to 2020. The mean PLL
148 calculated for the 121 individuals was 55.30 with a median of 59.38. Nine individuals recorded
149 PLLs below 10.0 with 1.09 being the lowest in only one individual. Seventy individuals
150 recorded PLLs over 50 with 100 recorded as the highest for five individuals. QTL analysis with
151 these data resulted in the detection of *Mfu10* (data not shown) but not *Mfu16*.

152

153



155 **Figure 3.** Significance and interaction between *Mfu10* and *Mfu16* determined by SAS GLIMMIX analysis

156

157 **Discussion**

158 We study the interaction of *M. fusca* (MAL0045) and derived progeny with different strains of
159 *E. amylovora* through artificial inoculation and QTL mapping. Through this process, *Mfu10*
160 was first identified on LG10 using Ea222 [9, 24]. The highly virulent Canadian strain, Ea3049,
161 affected *Mfu10* but did not overcome it, although this strain was not aggressive on MAL0045
162 [17, 19]. We therefore hypothesized a second putative resistance factor, possibly another locus,
163 might be involved in the resistance of MAL0045. The failure to detect another locus was partly
164 attributed to the fact that the first genetic map of MAL0045 was only scarce and did not
165 represent the whole genome [9, 24]. This hypothesis was predicated on the situation in Mr5
166 where, although one minor QTL was detected on LG5 after inoculation with Ea3049 [20], a
167 few more minor QTLs were detected following the development of a more saturated genetic
168 map and inoculation with different strains [22]. The first map of MAL0045 [9, 24], developed
169 with only the 05210 population, consists of 213 loci made up of DArT markers, a few SNPs
170 and SSRs developed from the apple genome [25]. On the other hand, using tunable genotyping-
171 by-sequencing technology (tGBS), thousands of *de novo* SNP markers were developed for
172 MAL0045, of which 560 SNPs were mapped including 53 SSR markers [19]. Thus, the dense
173 map has 400 markers more than the initial map and correctly represents the genome of
174 MAL0045. However, no minor fire blight locus were detected with this map [19]. Since fire
175 blight resistance is strain-dependent [21], we therefore speculated that the failure to detect a
176 minor locus is not only dependent on the marker density of the genetic map, but also the
177 interaction with the given *E. amylovora* strain used for inoculation.

178 Ea1038 phenotypic results showed that this mutant strain was as virulent as the wild type,
179 Ea3049 [17] with more than half of the 76 individuals having PLLs above 50 %. The mean PLL
180 of 61.7 with Ea1038 is similar to 62.4 recorded with Ea3049 on the 05210 population. The
181 results are also similar to the effect of Ea3049 on another wild apple *M. ×arnoldiana* –
182 MAL0004 [11], where the mean PLL recorded for 87 individuals was 65.9 %. It is interesting
183 to note that although this mutant is complemented with the C-allele, it was still very aggressive
184 to the individuals inoculated. This suggests that the switch from cysteine amino acid (C-allele)
185 to serine amino acid (S-allele) at position 156 of *avrRpt2EA* amino acid sequence [21] is not the
186 only factor that contributes to the pathogenicity of S-allele strains. Nevertheless, we detected
187 two QTLs of significant LODs on two different linkage groups, LG10 and LG16. It was quite
188 clear that *Mfu10* is QTL located on LG10, however, a novel minor fire blight QTL, never
189 previously detected with any strain was located on LG16 (*Mfu16*). We propose *Mfu16* as a
190 minor fire blight locus albeit unstable. Although, *Mfu16* was detected only with the subset of
191 76 individuals, and independently did not contribute significantly to resistance levels, it
192 positively affects *Mfu10*, as the effect of both loci is significantly stronger than *Mfu10* alone in
193 the 05210 individuals. Minor fire blight QTLs were detected on LGs 5, 7, 11, and 14 of Mr5
194 [22], however, only the minor QTL on LG7 was found to contribute to resistance in addition to
195 the major QTL on LG3 [10, 26].

196 Both the strain and the dense map were important factors in detecting *Mfu16* in this study. That
197 this mutant strain and not the wild type, Ea3049, led to the detection of *Mfu16* is indicative of
198 strong incompatible interaction between *M. fusca* and the C-allele of the *avrRpt2EA* effector of

199 *E. amylovora*. In addition, the failure to detect *Mfu16* in the initially developed map [9] is
200 indicative of the important role dense genetic maps play in molecular genetics studies in *Malus*
201 species and other plant species.

202 **Limitations**

203 The failure to detect *Mfu16* following the addition of more phenotypic data is somewhat of a
204 surprise and frankly unexplainable result, although we do not rule out the role of individuals in
205 the map with missing phenotypic data. Some of the individuals with missing phenotypic data
206 died off in the orchard and hence could not be phenotyped. It cannot be excluded that the
207 exchange of genotypes during scion cutting, grafting, inoculation or measuring could be a
208 reason, too. Nevertheless, the results are strong indications of a putative minor QTL on LG16
209 of MAL0045, which significantly improves the resistance of *Mfu10*. In the following years,
210 more phenotypic evaluation of these individuals and other established crosses with MAL0045-
211 derived progeny will help determine the usefulness and stability of *Mfu16*.

212

213 **Supplementary Information**

214 Not applicable.

215

216 **Abbreviations**

217 cfu/ml: colony forming units/milliliter; dpi: days post inoculations; GW: genome wide;
218 GLIMMIX: generalized linear mixed model; LG: linkage group; LOD: logarithm of the odds;
219 PLL: percent lesion length; QTL: quantitative trait locus; SNP: single nucleotide
220 polymorphisms; SSRs: simple sequence repeats;

221

222 **Ethics approval and consent to participate**

223 Not applicable

224

225

226 **Availability of data and material**

227 Data generated from this study are published within this article. Further materials can be
228 provided on request from the corresponding authors, Ofere Francis Emeriewen and Andreas
229 Peil.

230

231 **Funding**

232 Ofere Francis Emeriewen is funded by DFG (Deutsche Forschungsgemeinschaft) research
233 grant received – project number AOBJ 661177.

234

235 **Acknowledgements**

236 We acknowledge DFG for providing funding. We thank the orchard staff at Dresden-Pillnitz
237 and Quedlinburg for excellent technical assistance.

238

239 **Consent for publication**

240 We confirm that all authors read and approved this manuscript for publication

241

242 **Competing interests**

243 The authors' declare no competing interests

244

245

246 **Authors' contributions**

247 AP, OFE and MM for concept of the research, AP established the populations, OFE is
248 responsible for the project and AP supervises the research; AW developed the mutant strain,
249 KR performed the inoculations, AP and OFE performed mapping analyses, OFE and AP
250 prepared the manuscript, and all authors read and approved the manuscript.

251 **References**

252

253 1. Norelli JL, Jones AL, Aldwinckle HS. Fire blight management in the twenty-first
254 century: using new technologies that enhance host resistance in apple. *Plant Disease*.
255 2003 Jul;87(7):756-65. <https://doi.org/10.1094/PDIS.2003.87.7.756>

256 2. Malnoy M, Martens S, Norelli JL, Barny MA, Sundin GW, Smits TH, Duffy B. Fire
257 blight: applied genomic insights of the pathogen and host. *Annual review of
258 phytopathology*. 2012 Sep 8;50:475-94. <https://doi.org/10.1146/annurev-phyto-081211-172931>

260 3. Peil A, Emeriewen OF, Khan A, Kostick S, Malnoy M. Status of fire blight resistance
261 breeding in *Malus*. *Journal of Plant Pathology*. 2020 Jun 5:1-0.
262 <https://doi.org/10.1007/s42161-020-00581-8>

263 4. Emeriewen OF, Wöhner T, Flachowsky H, Peil A. *Malus* hosts–*Erwinia amylovora*
264 interactions: strain pathogenicity and resistance mechanisms. *Frontiers in plant science*.
265 2019 Apr 26;10:551. <https://doi.org/10.3389/fpls.2019.00551>

266 5. Yuan X, Hulin MT, Sundin GW. Effectors, chaperones, and harpins of the Type III
267 secretion system in the fire blight pathogen *Erwinia amylovora*: a review. *Journal of
268 Plant Pathology*. 2020 Aug 11:1-5. <https://doi.org/10.1007/s42161-020-00623-1>

269 6. Khan MA, Zhao YF, Korban SS. Molecular mechanisms of pathogenesis and resistance
270 to the bacterial pathogen *Erwinia amylovora*, causal agent of fire blight disease in
271 Rosaceae. *Plant Molecular Biology Reporter*. 2012 Apr 1;30(2):247-60. [DOI
272 10.1007/s11105-011-0334-1](https://doi.org/10.1007/s11105-011-0334-1)

273 7. Calenge F, Drouet D, Denancé C, Van de Weg WE, Brisset MN, Paulin JP, Durel CE.
274 Identification of a major QTL together with several minor additive or epistatic QTLs
275 for resistance to fire blight in apple in two related progenies. *Theoretical and Applied
276 Genetics*. 2005 Jun 1;111(1):128-35. [DOI 10.1007/s00122-005-2002-z](https://doi.org/10.1007/s00122-005-2002-z)

277 8. Durel CE, Denance C, Brisset MN. Two distinct major QTL for resistance to fire blight
278 co-localize on linkage group 12 in apple genotypes ‘Evereste’ and *Malus floribunda*
279 clone 821. *Genome*. 2009 Feb;52(2):139-47. <https://doi.org/10.1139/G08-111>

280 9. Emeriewen O, Richter K, Kilian A, Zini E, Hanke MV, Malnoy M, Peil A. Identification
281 of a major quantitative trait locus for resistance to fire blight in the wild apple species
282 *Malus fusca*. *Molecular Breeding*. 2014 Aug;34(2):407-19. [DOI 10.1007/s11032-014-0043-1](https://doi.org/10.1007/s11032-014-0043-1)

284 10. Peil A, Garcia-Libreros T, Richter K, Trognitz FC, Trognitz B, Hanke MV, Flachowsky
285 H. Strong evidence for a fire blight resistance gene of *Malus robusta* located on linkage
286 group 3. *Plant Breeding*. 2007 Oct;126(5):470-5. [doi:10.1111/j.1439-0523.2007.01408.x](https://doi.org/10.1111/j.1439-0523.2007.01408.x)

288 11. Emeriewen OF, Peil A, Richter K, Zini E, Hanke MV, Malnoy M. Fire blight resistance
289 of *Malus ×arnoldiana* is controlled by a quantitative trait locus located at the distal end
290 of linkage group 12. *European Journal of Plant Pathology*. 2017 Aug;148(4):1011-8.
291 [DOI 10.1007/s10658-017-1152-6](https://doi.org/10.1007/s10658-017-1152-6)

292 12. Parravicini G, Gessler C, Denance C, Lasserre-Zuber PA, Vergne E, Brisset MN,
293 Patocchi A, Durel CE, Broggini GA. Identification of serine/threonine kinase and
294 nucleotide-binding site–leucine-rich repeat (NBS-LRR) genes in the fire blight

295 resistance quantitative trait locus of apple cultivar 'Evereste'. Molecular plant
296 pathology. 2011 Jun;12(5):493-505. [DOI: 10.1111/J.1364-3703.2010.00690.X](https://doi.org/10.1111/J.1364-3703.2010.00690.X)

297 13. Fahrendrapp J, Broggini GA, Kellerhals M, Peil A, Richter K, Zini E, Gessler C. A
298 candidate gene for fire blight resistance in *Malus ×robusta* 5 is coding for a CC–NBS–
299 LRR. Tree genetics & genomes. 2013 Feb;9(1):237-51. [DOI 10.1007/s11295-012-0550-3](https://doi.org/10.1007/s11295-012-0550-3)

300 14. Emeriewen OF, Richter K, Piazza S, Micheletti D, Broggini GA, Berner T, Keilwagen
301 J, Hanke MV, Malnoy M, Peil A. Towards map-based cloning of *FB_Mfu10*:
302 identification of a receptor-like kinase candidate gene underlying the *Malus fusca* fire
303 blight resistance locus on linkage group 10. Molecular breeding. 2018 Aug;38(8):1-4.
304 <https://doi.org/10.1007/s11032-018-0863-5>

305 15. Emeriewen OF, Richter K, Flachowsky H, Malnoy M, Peil A. Genetic analysis and fine
306 mapping of the fire blight resistance locus of *Malus ×arnoldiana* on linkage group 12
307 reveal first candidate genes. Frontiers in Plant Science 2021 April
308 <https://doi.org/10.3389/fpls.2021.667133>

309 16. Broggini GA, Wöhner T, Fahrendrapp J, Kost TD, Flachowsky H, Peil A, Hanke MV,
310 Richter K, Patocchi A, Gessler C. Engineering fire blight resistance into the apple
311 cultivar 'Gala' using the FB _ MR 5 CC-NBS-LRR resistance gene of *Malus ×robusta*
312 5. Plant biotechnology journal. 2014 Aug;12(6):728-33. [doi: 10.1111/pbi.12177](https://doi.org/10.1111/pbi.12177)

313 17. Emeriewen OF, Richter K, Hanke MV, Malnoy M, Peil A. The fire blight resistance
314 QTL of *Malus fusca* (*Mfu10*) is affected but not broken down by the highly virulent
315 Canadian *Erwinia amylovora* strain E2002A. European journal of plant pathology. 2015
316 Mar;141(3):631-5. [DOI 10.1007/s10658-014-0565-8](https://doi.org/10.1007/s10658-014-0565-8)

317 18. Emeriewen OF, Richter K, Hanke MV, Malnoy M, Peil A. Further insights into *Malus*
318 *fusca* fire blight resistance. Journal of Plant Pathology. 2017 Oct 1:45-9.
319 <https://www.jstor.org/stable/45156718>

320 19. Emeriewen OF, Richter K, Berner T, Keilwagen J, Schnable PS, Malnoy M, Peil A.
321 Construction of a dense genetic map of the *Malus fusca* fire blight resistant accession
322 MAL0045 using tunable genotyping-by-sequencing SNPs and microsatellites.
323 Scientific reports. 2020 Oct 1;10(1):1-0. <https://doi.org/10.1038/s41598-020-73393-6>

324 20. Peil A, Flachowsky H, Hanke MV, Richter K, Rode J. Inoculation of *Malus×robusta* 5
325 progeny with a strain breaking resistance to fire blight reveals a minor QTL on LG5.
326 InXII International Workshop on Fire Blight 896 2010 Aug 16 (pp. 357-362).

327 21. Vogt I, Wöhner T, Richter K, Flachowsky H, Sundin GW, Wensing A, Savory EA,
328 Geider K, Day B, Hanke MV, Peil A. Gene-for-gene relationship in the host–pathogen
329 system *Malus ×robusta* 5–*Erwinia amylovora*. New Phytologist. 2013
330 Mar;197(4):1262-75. [doi: 10.1111/nph.12094](https://doi.org/10.1111/nph.12094)

331 22. Wöhner TW, Flachowsky H, Richter K, Garcia-Libreros T, Trognitz F, Hanke MV, Peil
332 A. QTL mapping of fire blight resistance in *Malus ×robusta* 5 after inoculation with
333 different strains of *Erwinia amylovora*. Molecular breeding. 2014 Jun;34(1):217-30.
334 [DOI 10.1007/s11032-014-0031-5](https://doi.org/10.1007/s11032-014-0031-5)

335 23. Van Ooijen JW. MapQTL® 5, Software for the mapping of quantitative trait loci in
336 experimental populations. Kyazma BV, Wageningen. 2004;63.

337

338 24. Emeriewen O, Malnoy M, Richter K, Kilian A, Hanke MV, Peil A. Evidence of a major
339 QTL for fire blight resistance in the apple wild species *Malus fusca*. In XIII International
340 Workshop on Fire Blight 1056 2013 Jul 2 (pp. 289-293). [doi:10.17660/ActaHortic.2014.1056.49](https://doi.org/10.17660/ActaHortic.2014.1056.49)

341 25. Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, Fontana
342 P, Bhatnagar SK, Troggio M, Pruss D, Salvi S. The genome of the domesticated apple
343 (*Malus × domestica* Borkh.). *Nature genetics*. 2010 Oct;42(10):833-9.

344 26. Peil A, Hübert C, Wensing A, Horner M, Emeriewen OF, Richter K, Wöhner T,
345 Chagné D, Orellana-Torrejon C, Saeed M, Troggio M, et al. Mapping of fire blight
346 resistance in *Malus × robusta* 5 flowers following artificial inoculation. *BMC plant
347 biology*. 2019 Dec;19(1):1-2. <https://doi.org/10.1186/s12870-019-2154-7>

348
349