

1 **Acibenzolar-S-methyl and resistance quantitative trait loci complement each**  
2 **other to control apple scab and fire blight**

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10

11 **Abstract**

12 Diversifying disease control methods is a key strategy to sustainably reduce pesticides.  
13 Plant genetic resistance has long been used to create resistant varieties. Plant  
14 resistance inducers (PRI) are also considered to promote crop health, but their  
15 effectiveness is partial and can vary according to the environment and the plant  
16 genotype. We investigated the putative interaction between intrinsic (genetic) and PRI-  
17 induced resistance in apple when affected by scab and fire blight diseases. A large F1  
18 mapping population was challenged by each disease after a pre-treatment with  
19 acibenzolar-S-methyl (ASM) and compared with the water control. Apple scab and fire  
20 blight resistance quantitative trait loci (QTLs) were detected in both conditions and  
21 compared. ASM exhibited a strong effectiveness in reducing both diseases. When  
22 combined, QTL-controlled and ASM-induced resistance acted complementarily to  
23 reduce the symptoms from 85% to 100% depending on the disease. In our conditions,  
24 resistance QTLs were only slightly or rarely affected by ASM treatment, despite their

25 probable implication in various stages of the resistance build-up. Implications of these  
26 results are discussed considering already known results, the underlying mechanisms,  
27 cross-protection of both types of resistance against pathogen adaptation, and practical  
28 application in orchard conditions.

29 Keywords: Intrinsic resistance, induced defense, *Malus domestica*, *Venturia*  
30 *inaequalis*, *Erwinia amylovora*

### 31 **Introduction**

32 Plant diseases threaten global agricultural production, leading to the extensive use of  
33 pesticides. Plant genetic resistance is considered as a major lever, and breeding for  
34 new resistant varieties is very active in most crop species. However resistance genes  
35 exert selection pressures on pathogens, triggering their evolution (McDonald and  
36 Linde, 2002). Diversifying and pyramiding resistance genes, especially when  
37 controlling both qualitative and quantitative resistance, is considered as a promising  
38 approach for achieving durable resistance (Pilet-Nayel et al., 2017). Such  
39 diversification is indeed expected to promote conflicting selection pressures on  
40 pathogen populations, which should constrain them to an evolutionary compromise  
41 limiting their development.

42 Reduced reliance on conventional pesticides can be also achieved by biocontrol (Pal  
43 and McSpadden Gardener, 2006; Burketova et al., 2015). Plant resistance inducers  
44 (PRIs, also called elicitors or plant defense activators) are part of biocontrol methods.  
45 They include a range of chemical (Bektas and Eulgem, 2015) or biological (Wiesel et  
46 al., 2014) stimulators able to activate plant defenses, without direct toxicity against  
47 pathogens (Oostendorp et al., 2001; Oliveira et al., 2016). PRIs provide most often  
48 partial resistance only and their performance are greatly influenced by abiotic and biotic  
49 factors including the pathogen and the plant genotype (Walters et al., 2013). However,

50 mechanisms underlying these numerous interactions are not clearly understood.  
51 Regarding the plant, comprehensive knowledge on genotype-PRI interactions could  
52 re-orientate plant breeding programs towards responsive genotypes or assist the  
53 choice of varieties to be deployed in practice, especially if PRIs are intended to be used  
54 in pest management programs. Combination of genetic resistance with PRIs are  
55 expected to multiply selection pressures on pathogen populations and thus limit their  
56 evolution, similarly to the combination of genetic resistance.

57 Apple (*Malus domestica*) is a major fruit tree species which requires a tremendous  
58 amount of pesticides in temperate climate conditions (MacHardy, 1996). Apple  
59 genotypes display a great variability of responses to apple scab, caused by the  
60 ascomycete fungus *Venturia inaequalis* and fire blight, caused by the bacterium  
61 *Erwinia amylovora*, two major diseases impacting orchards. Genetic mapping studies  
62 revealed numerous R genes and QTLs involved in resistance against apple scab  
63 (summarized in Khajuria et al., 2018) and fire blight (reviewed in Malnoy et al., 2012;  
64 Emeriewen et al., 2019), conferring partial to complete resistance to the diseases.  
65 Some of these loci are known to be strain-specific, with breakdown of resistance  
66 already demonstrated (Parisi, 1993; Caffier et al., 2010, 2015; Wöhner et al., 2014;  
67 Peil et al., 2020; Patocchi et al., 2020). The *Rvi6* gene, also called *Vf* from *Malus*  
68 *floribunda*, was one of the genes most widely used in apple breeding programs but  
69 was overcome as early as 1981 by *V. inaequalis* race 6 (Parisi, 1993). In apple, as in  
70 most plant species, quantitative resistance is considered more durable than qualitative  
71 resistance due to the multiple loci controlling selection pressure on pathogen  
72 populations (Parlevliet, 2002; Pilet-Nayel et al., 2017). However a slow erosion of some  
73 QTLs has already been demonstrated (Caffier et al., 2014, 2016).

74 Acibenzolar-S-methyl (ASM), a functional salicylic acid analog, is one of the most  
75 promising PRIs on several plant species (Gozzo and Faoro, 2013). It promotes  
76 systemic acquired resistance (SAR) and consequently the induction of various defense  
77 responses including pathogenesis-related proteins, leading to the protection of many  
78 species against a broad spectrum of pathogens (Romero et al., 2001; Assis et al.,  
79 2015; Matsuo et al., 2019; Youssef et al., 2019; Ishiga et al., 2020). On apple, several  
80 studies reported significant control of apple scab (Bengtsson et al., 2006, 2009;  
81 Marolleau et al., 2017) and fire blight (Brisset et al., 2000; Maxson-Stein et al., 2002;  
82 Hassan and Buchenauer, 2007; Abo-Elyousr et al., 2010; Shahini Sough et al., 2010;  
83 Dugé de Bernonville et al., 2014; Ćimović et al., 2015; Johnson et al., 2016; Marolleau  
84 et al., 2017), as well as the triggering of molecular defense response (Brisset et al.,  
85 2000; Ziadi et al., 2001; Maxson-Stein et al., 2002; Dugé de Bernonville et al., 2014;  
86 Warneys et al., 2018). Although significant, performance of ASM exhibits variability  
87 that remains to be understood for its practical use in the orchard. Among others, the  
88 genetic factor is still poorly investigated.

89 In this paper, we explored the added value of combining intrinsic (genetic) resistance  
90 against apple scab and fire blight with induced resistance conferred by ASM using a  
91 genetic mapping approach. QTLs composition in a segregating population was  
92 compared between ASM-treated and -untreated plants in order to highlight genetic  
93 determinants which could explain or interact with ASM performance.

## 94 **Materials and Methods**

95 **Plant material.** All experiments were performed with a F1 population referred as the  
96 'TxF progeny' and previously described in Laloi et al. (2016). The 267 individuals were  
97 derived from a controlled cross between TN10-8 and Fiesta, two genotypes partially  
98 resistant to apple scab. Fiesta is also partially resistant to fire blight. For each

99 experiment, up to ten replicates per individual were grafted and grown in greenhouse  
100 under semi-controlled growing conditions (23°C day/20°C night, humidity 40-80% and  
101 artificial light to complement natural light). Graftwood was collected from a  
102 conservatory orchard located at INRAE (Angers, France) and grafted on MM106 apple  
103 rootstock. The two parents and two susceptible control cultivars (Golden Delicious and  
104 Gala) were also included in each experiment in 10 replicates.

105 **Experimental design, treatment application and phenotypic data collection.** For  
106 each experiment, out of the ten, four replicates with active growing shoots per individual  
107 and treatment condition were chosen and distributed in each treatment group in  
108 randomized blocks in the greenhouse. Two days before inoculation, plants were  
109 sprayed with a solution of BION® 50 WG (50% ASM; Syngenta, Basel, Switzerland)  
110 (called ASM treatment) or with reverse osmosis water as control (called the water  
111 control). A water dilution of 0.2 g.L<sup>-1</sup> for *V. inaequalis*-inoculated plants and 0.4 g.L<sup>-1</sup>  
112 for *E. amylovora*-inoculated ones was applied. The commercial advice (0.4 g.L<sup>-1</sup>) was  
113 reduced for scab to maintain enough phenotypic variability across TxF progeny.

114 Two *V. inaequalis* isolates were used: the reference isolate EU-B04 (Origin : Belgium,  
115 host : Golden Delicious) previously described in Caffier et al. (2015) and Le Cam et al.  
116 (2019) and the isolate 09BCZ014 (Origin : France, host : TN10-8 x Prima progeny  
117 (individual E063), referred to as isolate 2557 in Laloï et al. (2016)). Monoconidial  
118 suspensions were prepared from diseased dry leaves at a concentration of 2.5 x 10<sup>5</sup>  
119 conidia.mL<sup>-1</sup> and, sprayed on grafted trees, incubated thereafter two days at 17°C  
120 under plastic sheet to maintain a high humidity, according to the conditions described  
121 by Caffier et al. (2010). The percentage of leaf surface exhibiting sporulating lesions  
122 was scored at 14, 21 and 28 days post-inoculation using the ordinal scale (0-7)

123 described in Calenge et al. (2004). Two experiments were performed with isolate EU-  
124 B04 (coded Vi-B04\_1 et Vi-B04\_2) and one with 09BCZ014 (coded Vi-Z14).

125 The reference strain CFPB1430 of *E. amylovora* from the French collection of  
126 phytopathogenic bacteria (Paulin and Samson, 1973) was used for inoculation. The  
127 bacterial suspension was prepared as described in Dugé de Bernonville et al. (2014)  
128 at  $10^8$  colony-forming units (CFU).mL<sup>-1</sup>. Growing shoots (>10cm) were inoculated by  
129 cutting the two youngest unrolled leaves with scissors previously soaked in the  
130 bacterial suspension. The length of necrosis developing on stem was measured at 7,  
131 14 and 21 days post-inoculation. The ratio between necrosis length and total shoot  
132 length was used as a severity score. Two experiments were performed (coded Ea-  
133 1430\_1 and Ea-1430\_2).

134 **Data analysis of phenotypic data.** Phenotypic data were analyzed separately for  
135 ASM treatment or the water control. The area under the disease-progress curve  
136 (AUDPC) was calculated as a quantitative summary of disease severity over time:

$$137 \text{ AUDPC} = \sum_{i=0}^2 \frac{y_i + y_{i+1}}{2} x(t_{i+1} - t_i)$$

138 where  $y_i$  is the disease score at the  $i^{\text{th}}$  day of observation and  $t_i$  the number of day post-  
139 inoculation at the  $i^{\text{th}}$  observation.

140 All statistical analyses were performed using R software (Dalgaard, 2010). AUDPC  
141 were fitted for environmental trend effects using SpATS package (Rodríguez-Álvarez  
142 et al., 2018) which estimates a Best Linear Unbiased Prediction (BLUP) for each  
143 individual. Broad-sense heritability of each trait for each treatment was also estimated  
144 with the function 'getHeritability' of the same package.

145 **Genotyping data and genetic maps construction.** DNA was extracted from fresh  
146 young leaf samples using oKtopure™ LGC Genomics robot and quantified with  
147 Hoechst protocol. The TxF progeny was genotyped with the Illumina Infinium 20K  
148 SNPs array (Bianco et al., 2014). Genotyping data were analyzed using  
149 GenomeStudio® Genotyping Module (V2.0, Illumina Inc., San Diego, California, USA)  
150 software with manual correction whenever necessary to improve dataset quality.  
151 Markers were filtered according to their parental segregation profile (ABxAA for the  
152 female map and AAxAB for the male map; markers in ABxAB were not considered).  
153 Then a linkage map was constructed for each parent with JoinMap 4.1 software using  
154 the Kosambi mapping function (Van Ooijen, 2006). Map quality was checked by  
155 comparison with the apple reference genetic map of Di Pierro et al. (2016) and an  
156 updated version of the map (Howard et al., submitted). The software BiomercatorV4.2  
157 (Arcade et al., 2004) was used to curate manually erroneous marker positions.  
158 Seventeen microsatellite (SSR) markers located in genomic regions where scab  
159 resistance QTLs were expected according to previous publications were added after  
160 genotyping of the TxF progeny (Supplementary Table S1).

161 **QTL mapping.** In order to compare the contribution of QTLs in resistance and in the  
162 interaction between genotypes and ASM, a mapping of QTLs was performed  
163 independently in water-control and ASM-treated populations. QTL analyses were  
164 conducted using the R/qtl package (Broman et al., 2003). Simple interval mapping  
165 (SIM) and composite interval mapping (CIM) were estimated using multiple imputation  
166 method and normal distribution model. Cofactors for CIM were determined from the  
167 best prediction model simulated with 'stepwise' function. LOD score threshold were  
168 determined using 1000 permutations to identify the statistically significant QTLs ( $\alpha =$   
169 0.05 genome-wide). LOD thresholds were about 5 for both scab and fire blight

170 experiments. Suggestive QTLs with LOD score between 3 and 5 were also considered.  
171 LOD score, 2-LOD support confidence interval (CI) and contribution of each QTL to  
172 the overall phenotypic variance (individual  $R^2$ ) were extracted from R/qtl analyses,  
173 together with the global QTL contribution (global  $R^2$ ). Individual and global  $R^2$  were  
174 calculated with the 'fitqtl' (for fitting a defined multiple-QTL model) function. Interactions  
175 between QTLs were studied by variance analysis using the genotyping data of each  
176 SNP closest to the peak of each QTL and were detailed by the 'effectplot' function.  
177 These results were used to define the model for the calculation of the global  $R^2$  with  
178 'fitqtl'. To adequately compare QTL effects between treatments by taking into account  
179 the overall phenotypic variation in each treatment, relative QTL effect (rEffect) were  
180 calculated for each QTL as follows:  $rEffect = \frac{1}{\sigma_B} \times \frac{|BLUP(AA) - BLUP(AB)|}{2}$ , where AA and  
181 AB are the allelic versions at the SNP closest to the QTL peak and  $\sigma_B$  is the standard  
182 deviation of BLUP (adjusted for all other significant QTLs effects than the QTL  
183 considered) for a given treatment condition. The confidence intervals at 5% of rEffects  
184 were simulated by bootstrapping with 5000 replicates using 'boot' function: when  
185 comparing rEffects of a given QTL between the water control and ASM treatment, the  
186 overlap of the confidence intervals was interpreted as if the effects were not  
187 significantly different. For simplicity, QTL alleles were then considered equivalent to  
188 SNP alleles at QTL peaks. Besides, a QTL detected at almost the same genomic  
189 position in various experiments were considered and named as the same QTL.

190 In Vi-B04 experiments, a clear bimodal distribution was observed (see results below).  
191 This distribution fitted with the strong effect of the major QTL (simply called qT1 here)  
192 detected on LG1 of TN10-8 after inoculation with this isolate (Calenge et al., 2004) or  
193 with a mixture of isolates including EU-B04 (Lê Van et al., 2012). For that reason, the  
194 TxF progeny was subdivided into two subsets of individuals according to the



195 presence/absence of the resistance allele at qT1 predicted by SNP data at the QTL  
196 peak. QTL analyses were then conducted on both sub-populations (further called qT1+  
197 and qT1- sub-populations). Conversely, isolate 09BCZ014 was shown to partially  
198 overcome the QTL qT1 by Laloï et al. (2016; isolate '2557'). QTL analysis was thus  
199 performed on the whole TxF progeny with the latter isolate.

200

## 201 **Results**

202 **Phenotypic variability.** Only experiments involving the most replicates per individual,  
203 Vi-B04\_1, Vi-Z14 and Ea-1430\_1 are presented here (the two additional experiments  
204 Vi-B04\_2 and Ea-1430\_2 are available as supplementary data).

205 A significant reduction of AUDPC was observed for both scab and fire blight after ASM  
206 treatment compared to the water control (Table 1). For the TxF progeny, AUDPC was  
207 reduced by 54% for Vi-B04\_1, 73% for Vi-Z14 and 50% for Ea-1430\_1. Similar  
208 reductions were observed for the two parents and susceptible controls (Figure 1). A  
209 large variation was observed among individuals regardless of the conditions, with  
210 AUDPC values ranging from 0 to 150 for Vi-B04\_1, from 0 to 75 for Vi-Z14 and from 1  
211 to 11 for Ea-1430\_1 (Figure 1). A higher coefficient of variation was observed for scab  
212 (CV=0.75 – 1.27) than for fire blight (CV=0.27 – 0.34; Table 1). The distributions were  
213 generally unimodal except for the water control in Vi-B04\_1 where a clear bimodal  
214 distribution was observed (Figure 1A), fitting with the expected effect of QTL qT1 (see  
215 Materials and Methods). Broad-sense heritabilities ( $h^2$ ) were generally high whatever  
216 the conditions, ranging from 0.63 for fire blight after ASM treatment to 0.94 for scab  
217 after the water control (Table 1). Adjusting the data for spatial effects resulted in a  
218 limited increase of heritability from 0.01 to 0.08 units compared to no adjustment (data  
219 not shown). Correlation coefficients (Cor) between the water control and ASM

220 treatment were intermediate to high (0.6 – 0.9) according to the experiments (Figure  
221 1). The range of AUDPC values observed in ASM-treated individuals increased as  
222 AUDPC values of the corresponding water-treated individuals increased, suggesting  
223 interactions between genotypes and treatments especially for the less resistant  
224 individuals (Figure 1). AUDPC mean values were lower in Vi-B04\_2 and Ea-1430\_2  
225 experiments but the variation of AUDPC was similar (Supplementary Table S2 and  
226 Supplementary Figure S1). The correlation coefficients between both Vi-B04  
227 experiments were 0.74 for the water control and 0.73 for ASM-treatment, while they  
228 were only 0.19 and 0.16 between both Ea\_1430 experiments, respectively.

229 **QTL detection for *V. inaequalis* isolate 'EU-B04'**. A total of 6245 SNPs was found  
230 polymorphic in the TxF progeny and one or both parents. After discarding 4433 SNPs  
231 polymorphic in both parents and redundant, two parental genetic maps of 17 linkage  
232 groups were constructed with 853 and 959 SNPs for TN10-8 and Fiesta, respectively.  
233 Both parental maps were merged in a single file for further detections of QTLs  
234 (Supplementary Table S3).

235 For the water control condition, a major QTL effect (LOD ~ 77;  $R^2 = 69.9\%$ ) was  
236 detected on LG1 together with three other significant QTLs on LG T13, F11 and F17  
237 and one suggestive on LG F12 when considering the whole TxF progeny  
238 (Supplementary Table S4, Figure 2). The presence of the former QTL (corresponding  
239 to qT1) was consistent with the bimodal distribution observed above. In the qT1- sub-  
240 population, four significant QTLs and one suggestive QTL were detected on linkage  
241 groups T13, F3, F11, F17 and F12, respectively (Table2, Figure 2). They explained  
242 from 3.7 to 29.0 % of phenotypic variation and their relative effect (rEffect) varied from  
243 0.32 (QTL qF12) to 0.66 (QTL qF17), compared to 0.85 for QTL qT1 in the whole TxF  
244 progeny (Figure 2). A significant interaction was found between QTLs qF11 and qF17

245 (Table 3). No AUDPC difference was detectable between both alleles of qF11 when  
246 the 'unfavorable' (i.e., susceptible) allele of qF17 (AA) was present (genotypic classes  
247 AA:AA and AB:AA with high AUDPC values at 149.6 and 146.2, respectively). With the  
248 'favorable' (i.e., resistant) allele of qF17, the 'favorable' allele of qF11 was on the  
249 contrary very efficient with AUDPC values decreasing from 131.3 to 99.5 for the  
250 combination of QTL alleles AA:AB, indicating a complementary relationship between  
251 'favorable' alleles of both QTLs. Altogether, QTLs on LG T1, T13, F11, F12 and F17  
252 explained 81.9% of phenotypic variation in the whole progeny, whereas the five QTLs  
253 including the QTL interaction explained 65.1% of phenotypic variation in the qT1- sub-  
254 population (Table 1). For ASM-treatment condition, QTL qT1 still exhibited a strong  
255 effect (LOD ~ 46;  $R^2 = 60\%$ ) in the whole TxF progeny (Supplementary Table S4,  
256 Figure 2) together with QTLs qF17 and qF11 as a suggestive QTL (Supplementary  
257 Table S4). All three explained 70.2% of the phenotypic variation. In the qT1- sub-  
258 population, only three out of the five QTLs detected in the water-control condition were  
259 detected (Table 2). QTLs qT13 and qF3 completely disappeared after ASM treatment  
260 with a LOD score lower than 0.2 at the SNP near to the peak of the same QTL detected  
261 in the water control and a non-significant effect for qF3 (Figure 2). The three other  
262 QTLs were detected at roughly the same positions on linkage groups as with the water  
263 control and the significant interaction between qF11 and qF17 was also found with the  
264 same pattern as above (Table 3). Their relative effects were not significantly different  
265 from the water control and were thus not significantly modulated by ASM treatment,  
266 despite much stronger LOD score and  $R^2$  for qF17 (21.2 and 39.9% with ASM  
267 treatment compared to 12.4 and 29.0% with the water control) and lower LOD score  
268 and  $R^2$  for qF11 (7.6 and 10.7% compared to 10.7 and 18.1%, respectively) (Table 2,  
269 Figure 2).

270 For experiment Vi-B04\_2, the same QTLs were detected despite at lower LOD scores  
271 than in the first experimentation (Supplementary Table S5). In the whole TxF progeny,  
272 qT1 was still detected as the strongest QTL for both treatment conditions (LOD ~ 35;  
273  $R^2$  ~40-48%) together with qF11 and qF12 as suggestive QTL (water control) and qF17  
274 (both conditions) and still a significant interaction between qF11 and qF17  
275 (Supplementary Table S6, water control). In the qT1- sub-population, qF17 was the  
276 single significant QTL detected, while qT13, qF3, qF11 and qF12 were detected as  
277 suggestive QTLs with mostly similar  $R^2$  compared to the Vi-B04\_1 experiment. Three  
278 significant interactions between QTLs were detected, two of them involving qT13 with  
279 qF11 and qF17 respectively (Supplementary Table S6). In this experiment, ASM  
280 treatment did not significantly modulate the QTL effect on any of the QTLs detected  
281 (Supplementary Figure S2).

282 **QTL detection for *V. inaequalis* isolate '09BCZ14'**. For the water control condition,  
283 all the QTLs detected in Vi-B04\_1 were also detected in Vi-Z14, except on LG F12,  
284 and a new suggestive QTL was detected on LG T10. The phenotypic variation  
285 explained by these QTLs ranged from 3.5 to 10.7% and the relative effects from 0.25  
286 (QTL qT10) to 0.42 (QTLs qT13 and qF17) (Table 2, Figure 2). Interaction between  
287 QTLs qF11 and qF17 was still significant whereas new interactions were found  
288 significant between qT1 and qF3, between qT13 and qF3 and between qT10 and qF11  
289 (Table 3). Altogether, these QTLs and interactions explained 47.5% of the phenotypic  
290 variation (Table 1). For ASM-treatment condition, three out of the six QTLs identified  
291 in the water control were still detected but only as suggestive QTLs (qT13, qF11, qF17;  
292 Table 2). Altogether, they explained only 15.0% of the phenotypic variation without any  
293 interactions. The relative effects of these QTLs were not significantly different when

294 compared with the water control (Figure 2). Conversely, the relative effect of qT1 was  
295 significantly lower for ASM treatment than for the water control (Figure 2).

296 **QTL mapping for fire blight resistance.** For the water control condition, four QTLs  
297 (two significant and two suggestive) were detected on LG T3, T5, F7 and F15  
298 explaining from 3.1 to 20.1% of total phenotypic variation (Table 2, Figure 3).  
299 Altogether, these QTLs explained 35.0 % of the phenotypic variation. The same QTLs  
300 except LG F15 were found for ASM-treatment condition, together with a new significant  
301 QTL on LG F5 (Table 2). These QTLs explained from 4.6 to 13.2% of the phenotypic  
302 variation (33.1% altogether). On LG T5, the peak of the QTLs was offset by ~20cM  
303 between the water control and ASM treatment, which suggests two different QTLs. The  
304 relative effects of these QTLs were not significantly different when compared with the  
305 water control (Figure 3). No significant interaction could be identified between the QTLs  
306 in both conditions.

307 In the Ea-1430\_2 experiment for water-control condition, three QTLs previously  
308 identified were maintained (qT3, qT5, qF15), two new QTLs were detected (qT15, qF5)  
309 and one was no longer detected (qF7) (Supplementary Table S5). Surprisingly, qF7  
310 was the QTL with the strongest effect in the first experiment; only a weak peak was  
311 detected here with a LOD of 1.8. For ASM treatment condition, three new QTLs qT1,  
312 qT7 and qF1 were identified while three others disappeared (qT3, qT15, qF15). The  
313 QTL interactions in the two conditions are totally different (Supplementary Table S6).  
314 Among the nine QTLs identified, qT3 and qF15 relative effect were significantly  
315 modulated by ASM (Supplementary Figure S3).

## 316 **Discussion**

317 A first result of our study has been to confirm already known scab and fire blight  
318 resistance QTLs and to detect new ones under the water control condition. For scab

319 resistance, QTLs located on LG1 of TN10-8 (qT1) and on LG11 and LG17 of Fiesta  
320 (qF11 and qF17, respectively) were previously detected and confirmed in various  
321 studies (Durel et al., 2003; Liebhard et al., 2003; Calenge et al., 2004; Soufflet-Freslon  
322 et al., 2008; Lê Van et al., 2012). The added value of the present study is the refinement  
323 of their genetic position with reduced confidence intervals compared to previous  
324 studies. As stated above, qT1 is precisely colocalized with the *Rvi6* (*Vf*) gene at  
325 position 43.03 cM of the genetic map corresponding to the CH-Vf1 SSR marker, tightly  
326 associated to that R-gene (Vinatzer et al., 2004). qT1 is thus a potential allele or  
327 paralog of *Rvi6* and can thus be postulated as an extracellular leucine-rich repeat  
328 receptor-like gene (Belfanti et al., 2004; Calenge et al., 2004). Another key aspect is  
329 the confirmation of the strong complementary (synergistic) interaction between qF11  
330 and qF17 as already highlighted by Caffier et al. (2014). Such an interaction can be  
331 interpreted as the complementary action of two genes successively involved in a  
332 metabolic pathway (McMullen et al., 1998). The dependency of the qF11 allelic  
333 contrast upon the unfavorable or favorable allele of qF17 could be tentatively  
334 interpreted as an upstream position of the gene underlying qF17 and a downstream  
335 position of the gene underlying qF11. Thus, qF11 and qF17 could rather be involved  
336 in a signaling or defense pathway, unlike qT1 more probably involved in pathogen  
337 effector or PAMP recognition. Although reduced, the confidence intervals of these  
338 QTLs were still too wide to search for sufficiently plausible candidate genes. An attempt  
339 was made for qF17 and yielded over 160 positional candidates (data not shown). New  
340 scab resistance QTLs were detected on LGs 10 and 13 of TN10-8 and LGs 3 and 12  
341 of Fiesta. The most significant were qT13 and qF3 detected at genomic positions not  
342 yet identified in other scab resistance studies. They will therefore be considered as a  
343 priority for further marker-assisted breeding.

344 For fire blight resistance, the QTL qF7 was consistently detected on LG7 of Fiesta in  
345 the first experiment as already published (Calenge et al., 2005; Khan et al., 2007). The  
346 refinement of its genetic position was allowed with reduced confidence intervals  
347 compared to previous studies. This QTL was shown to interact with two other ones on  
348 LG8 and LG13 (van de Weg et al., 2018), but these genomic regions were not detected  
349 in our study. Implication in a metabolic pathway could nevertheless be postulated for  
350 qF7 due to such interactions. We also identified resistance QTLs on LGs 3, 5 and 15  
351 which may coincide with those published by Calenge et al. (2005), Durel et al. (2009)  
352 and Le Roux et al. (2010) respectively, despite their genomic position was not very  
353 accurate. In our second experiment, qF7 was no more detected which calls into  
354 question the relevance of this experiment, especially in view of the low AUDPC and  
355 heritability values obtained and the weak correlation between both Ea-1430  
356 experiments. This second experiment was performed very early in the season (early  
357 February) at an unusual time for fire blight experiment which could explain this  
358 surprising result.

359 In the present study, we propose for the first time a QTL mapping approach to search  
360 for genetic factors influenced by a PRI. A few studies highlighted the influence of  
361 genotypes on the effectiveness of PRIs but without any link with intrinsic genetic  
362 resistance of varieties (Vallad and Goodman, 2004; Sharma et al., 2010; Maisonneuve  
363 et al., 2013). Pawlowski et al. (2016) underlined the specific interaction between the  
364 genetic and PRI-induced resistance but did not characterize the genetic resistance  
365 factors. A mapping approach has the advantage of exploring in detail the putative  
366 modulation of genetic resistance factors by PRIs. In our study, the results show that  
367 genetic resistance controlled by QTLs is only slightly or rarely affected by ASM  
368 treatment. Out of the seven scab resistance QTLs detected, only two QTLs (qF3 for

369 Vi-B04\_1, and qT1 for Vi-Z14) almost disappeared after treatment with ASM. We also  
370 detected two fire blight resistance QTLs (qT3, qF15) which effects significantly  
371 decreased after ASM treatment in the second Ea-1430 experiment, but the  
372 experimental conditions were questionable. Despite a large mapping population, the  
373 confidence intervals surrounding relative effects of QTLs were large and therefore  
374 precluded the detection of low modulation of QTL effects by ASM. More individuals  
375 and replicates per individual would have been necessary to detect such modulations.  
376 Furthermore, no QTL specific to the ASM treatment condition was detected: all QTLs  
377 detected with ASM were already identified as resistance QTLs under the water control.  
378 The general trend was either a maintenance or a reduction in the relative effect of  
379 QTLs. Some of them fully disappeared (significantly) but this observation was hard to  
380 repeat for both diseases. To some extent, this QTL effect disappearance could also be  
381 explained by a scale effect. Indeed, the application of ASM strongly reduces the global  
382 variability in disease severity and the weakest QTLs under the water control can  
383 become insignificant under ASM treatment due to a consequent lack of statistical  
384 power. However, a general trend of maintenance or small reduction of most QTLs is  
385 surprising: considering the overall effectiveness of ASM on reducing disease  
386 symptoms and the interactions observed between less resistant genotypes and  
387 treatment, it would have been expected to found more interactions between pathways  
388 leading to this resistance.

389 To go further, according to the diverse nature of the QTLs present in our population,  
390 we expected to observe some QTLs responding to ASM and others remaining  
391 unchanged. ASM is known to act on salicylic acid signaling pathway. In tobacco, it is  
392 perceived by the SABP2 protein (Salicylic acid-binding protein 2) which transforms it  
393 into acibenzolar which then interacts with NPR1 to activate the transcription of a set of



394 genes in the SAR pathway (Tripathi et al., 2010; Warneys et al., 2018; De Jong et al.,  
395 2019; Ishiga et al., 2020; Li et al., 2020). If we consider the three stages of resistance  
396 build-up, namely recognition, signaling and defense, we could postulate that QTLs  
397 involved in signaling and defense could be more affected by ASM than QTLs involved  
398 in effectors or PAMPs recognition. However, the qT1 previously described as most  
399 probably involved in pathogen recognition disappeared with 09BCZ014 isolate but not  
400 with EU-B04, whereas the QTLs qF11, qF17 and qF7, potentially rather involved in  
401 signaling or defense pathways were not significantly affected by ASM. Various  
402 hypotheses can be formulated for interpreting our results. In Arabidopsis, Tsuda et al.  
403 (2009) showed that each of the salicylic acid (SA), jasmonic acid (JA) and ethylene  
404 (ET) signaling sectors can positively contribute to immunity against both biotrophic and  
405 necrotrophic pathogens. The authors schematized various situations of compensation  
406 or synergy between different sectors of a complex signaling network where the effector  
407 or PAMP recognition (input) can generate equivalent restriction of pathogen growth  
408 (output) while mobilizing very different sectors interacting or not. In our situation, ASM  
409 treatment would reinforce one of the signaling sectors with possible consequences on  
410 other sectors given SA *versus* JA/ET compensation, while resistance QTLs positioned  
411 at various locations of the network would impact the flow along some of these  
412 pathways. Some of the detected QTLs could therefore be located on metabolic  
413 pathways independent of the SA pathway while others could be implied in the SA  
414 pathway and thus be affected by the strong initial supply of ASM. In-depth exploration  
415 of the metabolic mechanisms involved in genotypes carrying or not resistance QTL  
416 alleles, with or without ASM treatment, is needed to precise the interactions between  
417 pathways.

418 Our study revealed that the intrinsic resistance controlled by QTLs and the resistance  
419 induced by ASM have a complementary effect for the control of apple scab and fire  
420 blight. In our study, the average effectiveness of ASM in reducing the disease ranged  
421 from 50% for Ea-1430\_1 to 78% for V1-B04\_2. Under the water control, the individuals  
422 cumulating favorable alleles at all detected QTLs exhibited a disease reduction of 91%  
423 for Vi-B04\_1 and 98% for Vi-Z14 compared to individuals carrying only unfavorable  
424 QTL alleles for scab, and 78% for fire blight. When intrinsic and ASM-induced  
425 resistance was combined, the disease reduction was close to 100% for scab and 85%  
426 for fire blight. These results confirm that there is no incompatibility between intrinsic  
427 and ASM-induced resistance for apple as reported for cucumber (da Rocha and  
428 Hammerschmidt, 2005). The present results were obtained under controlled conditions  
429 but arguments suggest that the same trends could be observed in orchards. On the  
430 one hand, Caffier et al. (2014, 2016) showed that quantitative resistance related to  
431 QTLs qT1, qF11 and qF17 significantly reduced scab severity in orchards. On the other  
432 hand, Marolleau et al. (2017) confirmed that ASM could be integrated into orchard  
433 protection practices to control apple scab. Combining both types of resistance in the  
434 orchard should allow a better control of apple scab and similarly for fire blight.

435 In addition to improve protection effectiveness, the interest in combining intrinsic and  
436 PRI-induced resistance could rely in a cross-protection of both types of resistance  
437 through a diversification of selection pressures on pathogen populations which would  
438 reduce or slow down their adaptation (Lê Van et al., 2013; Caffier et al., 2014). The  
439 use of ASM in combination with genotypes cumulating several QTLs involved in  
440 different defense pathways could be a promising sustainable protection for the control  
441 of apple scab and fire blight. A thorough analysis of the mechanisms activated by this

442 resistance will confirm these hypotheses and all of these results must be confirmed in  
443 the orchards for possible integration into the protection practices.

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751 **Tables**

752 **Table 1.** Mean values ( $\pm$  standard-deviation) of AUDPC, relative effectiveness of ASM, coefficient of variation of individual BLUPs  
 753 (CV), broad-sense heritability ( $h^2$ ) and phenotypic variation explained by all detected resistance QTLs (global  $R^2$ ) against scab (*V.*  
 754 *inaequalis* isolates EU-B04 and 09BCZ14; experiments Vi-B04\_1 and Vi-Z14, respectively) and fire blight (*E. amylovora* isolate  
 755 CFPB1430; experiment Ea-1430\_1) in the TxF progeny. Values for the sub-population qT1- are indicated within brackets.  
 756 Effectiveness of ASM is computed as: (Mean-water – Mean-PRI)/Mean-water.

757

Experiment	Treatment	Mean	Effectiveness ASM (%)	CV	$h^2$	Global $R^2$
Vi-B04_1	water	41.2 $\pm$ 36.8		0.84 [0.29] <sub>qT1-</sub>	0.94	81.9 [65.1] <sub>qT1-</sub>
	ASM	18.9 $\pm$ 26.4	54	1.27 [0.48] <sub>qT1-</sub>	0.91	70.2 [53.8] <sub>qT1-</sub>
Vi-Z14	water	24.1 $\pm$ 21.4		0.75	0.84	47.5
	ASM	6.6 $\pm$ 8.4	73	0.88	0.71	15.0
Ea-1430_1	water	6.2 $\pm$ 2.4		0.27	0.70	35.0
	ASM	3.1 $\pm$ 1.8	50	0.34	0.63	33.1

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759

760 **Table 2.** Parameters associated with the quantitative trait loci (QTLs) identified for disease resistance against scab (experiments Vi-  
761 B04\_1, sub-population qT1-, and Vi-Z14) and fire blight (experiment Ea-1430\_1) in the TxF progeny after water- or ASM-treatment.

762

763 <sup>a</sup> LOD thresholds were obtained after permutations (n=1000): 5.1 (Vi-B04<sub>qT1-</sub>\_water), 4.6 (Vi-B04<sub>qT1-</sub>\_ASM), 5.0 (Vi-Z14\_water and  
764 Vi-Z14\_ASM), 4.7 (Ea-1430\_water) and 4.6 (Ea-1430\_ASM). Significant QTLs are shown in bold. Suggestive QTLs with LOD score  
765 between 3.0 and the LOD threshold are shown in italics. QTLs being insignificant in a condition but significant in another are shown  
766 in grey.

767 <sup>b</sup> position of 2-LOD support QTL confidence interval borders

768

Trait	Treatment	Linkage group	Position	LOD <sup>a</sup>	R <sup>2</sup>	CI <sup>b</sup>
Vi-B04_1 <sub>qT1-</sub>	water	<b>T13</b>	<b>3.58</b>	<b>8.6</b>	<b>9.3</b>	<b>0.00 – 6.80</b>
		<b>F3</b>	<b>13.36</b>	<b>5.7</b>	<b>5.5</b>	<b>8.25 – 18.54</b>
		<b>F11</b>	<b>5.00</b>	<b>10.7</b>	<b>18.1</b>	<b>0.00 – 20.98</b>
		<i>F12</i>	25.99	3.0	3.7	20.46 – 31.56
		<b>F17</b>	<b>18.92</b>	<b>12.4</b>	<b>29.0</b>	<b>16.52 – 27.35</b>
	ASM	<i>T13</i>	3.58	0.3	-	-
		<i>F3</i>	44.80	0.8	-	-
		<b>F11</b>	<b>0.00</b>	<b>7.6</b>	<b>10.7</b>	<b>0.00 – 7.91</b>
		<i>F12</i>	19.28	4.0	5.1	10.83 – 24.43
		<b>F17</b>	<b>18.00</b>	<b>21.2</b>	<b>39.9</b>	<b>16.52 – 20.90</b>
Vi-Z14	water	<b>T1</b>	<b>45.03</b>	<b>9.4</b>	<b>10.7</b>	<b>39.01 – 52.89</b>
		<i>T10</i>	62.09	3.6	3.5	54.88 – 65.69
		<b>T13</b>	<b>3.18</b>	<b>10.9</b>	<b>10.1</b>	<b>0.00 – 6.80</b>
		<b>F3</b>	<b>3.92</b>	<b>5.1</b>	<b>5.3</b>	<b>0.00 – 9.03</b>
		<b>F11</b>	<b>5.53</b>	<b>7.8</b>	<b>7.9</b>	<b>0.00 – 20.98</b>
	ASM	<b>F17</b>	<b>18.92</b>	<b>9.1</b>	<b>10.7</b>	<b>14.18 – 27.35</b>
		<i>T1</i>	36.61	1.7	-	-
		<i>T10</i>	62.09	2.2	-	-
		<i>T13</i>	0.00	4.1	6.3	0.00 – 6.80
		<i>F3</i>	5.50	1.1	-	-
Ea-1430_1	water	<i>F11</i>	3.17	3.9	5.0	0.00 – 8.69
		<i>F17</i>	18.92	4.2	5.0	13.79 – 27.35
		<i>T3</i>	23.57	4.1	3.1	11.81 – 26.76
		<b>T5</b>	<b>54.33</b>	<b>5.8</b>	<b>5.2</b>	<b>43.24 – 62.05</b>
		<i>F5</i>	39.73	2.5	-	-
	ASM	<b>F7</b>	<b>69.46</b>	<b>18.4</b>	<b>20.1</b>	<b>67.10 – 71.42</b>
		<i>F15</i>	76.05	3.7	3.9	70.94 – 81.15
		<i>T3</i>	20.38	3.7	4.6	17.13 – 29.18
		<b>T5</b>	<b>35.22</b>	<b>4.9</b>	<b>4.6</b>	<b>28.37 – 40.84</b>
		<b>F5</b>	<b>38.56</b>	<b>6.3</b>	<b>8.4</b>	<b>32.18 – 42.08</b>
	<b>F7</b>	<b>69.07</b>	<b>12.2</b>	<b>13.2</b>	<b>67.10 – 71.42</b>	
	<i>F15</i>	86.70	2.9	-	-	

770 **Table 3.** Interactions between significant and suggestive QTLs identified in Vi-B04\_1<sub>qT1-</sub> and Vi-Z14 experiments.

771

		water					ASM				
Trait	QTLs combination	AA:AA	AB:AA	AA:AB	AB:AB	F-value <sup>a</sup>	AA:AA	AB:AA	AA:AB	AB:AB	F-value <sup>a</sup>
Vi-B04_1 <sub>qT1-</sub>	qF11:qF17	149.6	146.2	99.5	131.3	12.1 ***	94.7	93.2	49.0	70.0	8.9 **
Vi-Z14	qT1:qF3	34.2	19.2	24.7	16.6	6.6 *	-	-	-	-	-
	qT13:qF3	19.9	35.5	15.8	25.4	6.6 *	-	-	-	-	-
	qT10:qF11	15.7	24.3	23.1	31.2	4.9 *	-	-	-	-	-
	qF11:qF17	31.3	32.3	12.8	24.9	4.2 *	-	-	-	-	-

772

773 <sup>a</sup> F-value of the variance analysis Fisher-test where stars represent the significance of the test at the risk of 0.05 (\*), 0.01 (\*\*) or  
774 0.005 (\*\*\*).

775 **Figures Captions**

776 **Figure 1.** Relationship between disease severity (BLUP estimates) for water- and  
777 ASM-treated individuals of the TxF progeny in (A) Vi-B04\_1, (B) Vi-Z14 and (C) Ea-  
778 1430 experiments. Distributions of BLUPs are shown in the upper (water control) and  
779 right (ASM treatment) part of each graph where the complete population is represented  
780 in grey and the sub-population qT1- (i.e., individuals selected as not-carrying the  
781 resistance allele of the major QTL qT1) in green. The same color code is used in the  
782 graph where the green dots are superimposed on the grey dots. Control and parental  
783 genotypes are shown with triangles in orange (TN10-8), red (Fiesta) and black (Gala  
784 and Golden Delicious). Cor is the Pearson coefficient of correlation between water and  
785 ASM BLUPs. Values for the sub-population qT1- are indicated within brackets.

786 **Figure 2.** LOD score curves of QTLs involved in resistance against scab (Vi-B04\_1  
787 and Vi-Z14) for each treatment identified in the TxF progeny (upper part) and relative  
788 effects (rEffects) of these QTLs (lower part). Blue and orange colors represent  
789 respectively the water control and ASM treatment. LOD score thresholds are drawn as  
790 horizontal lines with the respective colors. In each rEffect plot, mean and standard  
791 deviation of each rEffect, calculated with bootstrapping, are represented by a point and  
792 a vertical bar respectively, with the respective blue and orange color for water-control  
793 and ASM-treatment respectively.

794 **Figure 3.** LOD score curves of QTLs involved in resistance against fire blight (Ea-  
795 1430\_1) for each treatment identified in the TxF progeny (upper part) and relative  
796 effects (rEffects) of these QTLs (lower part). Blue and orange colors represent  
797 respectively the water control and ASM treatment. LOD score thresholds are drawn as  
798 horizontal lines with the respective colors. In each rEffect plot, mean and standard  
799 deviation of each rEffect, calculated with bootstrapping, are represented by a point and

800 a vertical bar respectively, with the respective blue and orange color for water-control  
801 and ASM-treatment respectively.



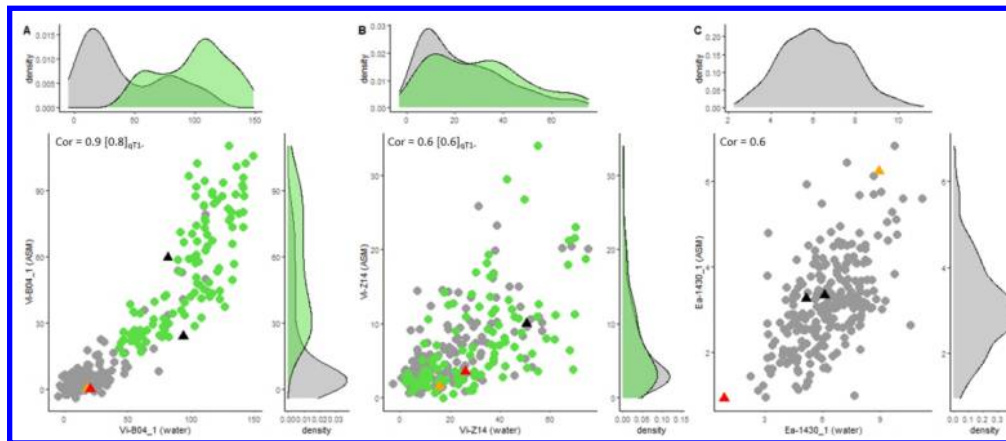


Figure 1. Relationship between disease severity (BLUP estimates) for water- and ASM-treated individuals of the TxF progeny in (A) Vi-B04\_1, (B) Vi-Z14 and (C) Ea-1430 experiments. Distributions of BLUPs are shown in the upper (water control) and right (ASM treatment) part of each graph where the complete population is represented in grey and the sub-population qT1- (i.e., individuals selected as not-carrying the resistance allele of the major QTL qT1) in green. The same color code is used in the graph where the green dots are superimposed on the grey dots. Control and parental genotypes are shown with triangles in orange (TN10-8), red (Fiesta) and black (Gala and Golden Delicious). Cor is the Pearson coefficient of correlation between water and ASM BLUPs. Values for the sub-population qT1- are indicated within brackets.

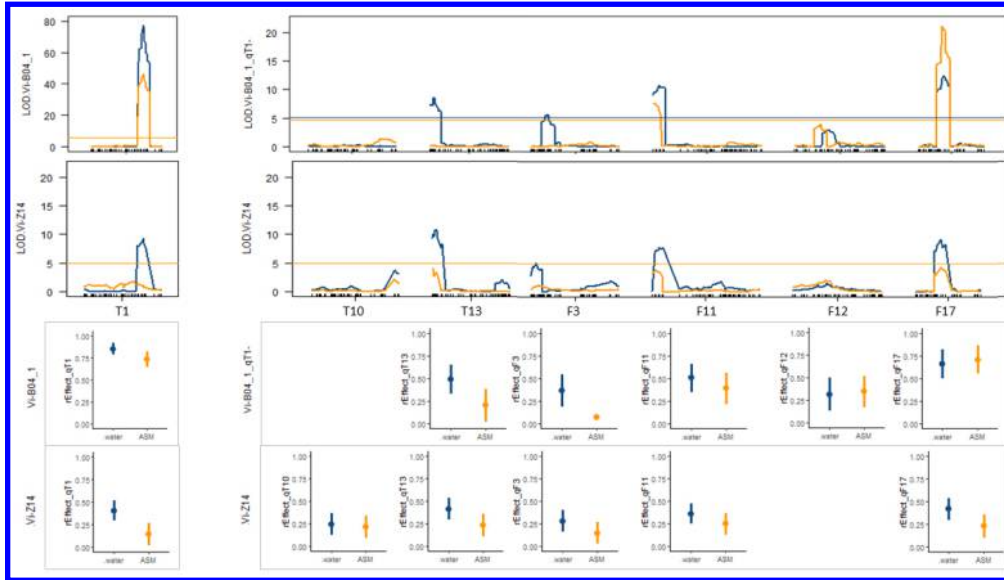


Figure 2. LOD score curves of QTLs involved in resistance against scab (Vi-B04\_1 and Vi-Z14) for each treatment identified in the TxF progeny (upper part) and relative effects (rEffects) of these QTLs (lower part). Blue and orange colors represent respectively water control and ASM treatment. LOD score thresholds are drawn as horizontal lines with the respective colors. In each rEffect plot, mean and standard deviation of each rEffect, calculated with bootstrapping, are represented by a point and a vertical bar respectively, with the respective blue and orange color for water-control and ASM-treatment respectively.

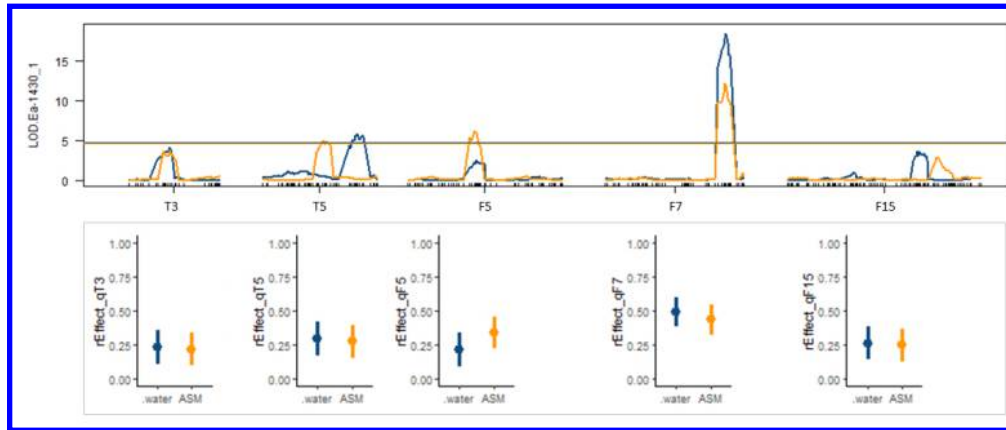


Figure 3. LOD score curves of QTLs involved in resistance against fire blight (Ea-1430\_1) for each treatment identified in the TxF progeny (upper part) and relative effects (rEffects) of these QTLs (lower part). Blue and orange colors represent respectively water control and ASM treatment. LOD score thresholds are drawn as horizontal lines with the respective colors. In each rEffect plot, mean and standard deviation of each rEffect, calculated with bootstrapping, are represented by a point and a vertical bar respectively, with the respective blue and orange color for water-control and ASM-treatment respectively.

**Supplementary Table S1. List of microsatellite markers added to the genetic map**

Marker Name	Marker type	Linkage group	Reference
CH05g08	SSR	T1	(Liebhard et al., 2002)
CH-Vf1	SSR	T1	(Vinatzer et al., 2004)
HB04p	SSR	T1	(Broggini et al., 2009)
Hi02c07	SSR	T1	(Silfverberg-Dilworth et al., 2006)
KA4b	SSR	T1	(Yamamoto et al., 2004)
Md-Exp7	SSR	T1	(Costa et al., 2008)
NZmsCN879773	SSR	T1	(Celton et al., 2009)
Vf2ARD	SCAR	T1	(Boudichevskaia et al., 2009)
CH02d08	SSR	F11	(Liebhard et al., 2002)
CH04h02	SSR	F11	(Liebhard et al., 2002)
Rvi18SSR	SSR	F11	(Soriano et al., 2014)
CH01h01	SSR	F17	(Liebhard et al., 2002)
CH04f08	SSR	F17	(Liebhard et al., 2002)
CH05g03	SSR	F17	(Liebhard et al., 2002)
GD96	SSR	F17	(Hokanson et al., 1998)
Hi02f12	SSR	F17	(Silfverberg-Dilworth et al., 2006)
Hi03c05	SSR	F17	(Silfverberg-Dilworth et al., 2006)

**Supplementary Table S2.** Mean values ( $\pm$  standard-deviation) of AUDPC, relative effectiveness of ASM, coefficient of variation of individual BLUPs (CV), broad-sense heritability ( $h^2$ ) and phenotypic variation explained by all detected resistance QTLs (global  $R^2$ ) against scab (*V. inaequalis* isolate EU-B04; experiment Vi-B04\_2) and fire blight (*E. amylovora* isolate CFPB1430; experiment Ea-1430\_2) in the TxF progeny. Values for the sub-population qT1- are indicated within brackets. Effectiveness of ASM is computed as: (Mean-water – Mean-PRI)/Mean-water.

Experiment	Treatment	Mean	Effectiveness ASM (%)	CV	$h^2$	Global $R^2$
Vi-B04_2	water	22.9 $\pm$ 26.2		1.03 [0.47] <sub>qT1-</sub>	0.89	56.1 [68.8] <sub>qT1-</sub>
	ASM	5.1 $\pm$ 8.9	78	1.62 [0.59] <sub>qT1-</sub>	0.85	56.3 [56.7] <sub>qT1-</sub>
Ea-1430_2	water	4.2 $\pm$ 2.5		0.30	0.51	30.2
	ASM	2 $\pm$ 1.5	52	0.43	0.56	26.1

**Supplementary Table S4.** Parameters associated with the quantitative trait loci (QTLs) identified for disease resistance against scab (experiment Vi-B04\_1, whole population) in the TxF progeny after water- or ASM-treatment.

Trait	Treatment	Linkage group	Position	LOD <sup>a</sup>	R <sup>2</sup>	CI <sup>b</sup>
Vi-B04_1	water	<b>T1</b>	<b>43.00</b>	<b>77.1</b>	<b>69.9</b>	<b>41.01 – 44.23</b>
		<b>T13</b>	<b>3.58</b>	<b>6.3</b>	<b>2.8</b>	<b>0.00 – 6.80</b>
		F3	12.19	1.6	-	-
		<b>F11</b>	<b>6.00</b>	<b>8.9</b>	<b>7.7</b>	<b>0.00 – 20.98</b>
		<i>F12</i>	<i>4.00</i>	<i>3.3</i>	<i>0.6</i>	<i>0.00 – 6.87</i>
		<b>F17</b>	<b>18.92</b>	<b>16.7</b>	<b>12.8</b>	<b>16.52 – 20.90</b>
	ASM	<b>T1</b>	<b>43.03</b>	<b>46.1</b>	<b>60.0</b>	<b>41.01 – 44.23</b>
		T13	3.58	0.8	-	-
		F3	1.96	0.3	-	-
		<i>F11</i>	<i>4.75</i>	<i>4.3</i>	<i>6.9</i>	<i>0.00 – 20.98</i>
		F12	19.28	2.3	-	-
		<b>F17</b>	<b>18.92</b>	<b>15.9</b>	<b>24.9</b>	<b>16.52 – 20.90</b>

<sup>a</sup> LOD thresholds were obtained after permutations (n=1000): 5.5 (Vi-B04\_water) and 5.4 (Vi-B04\_ASM). Significant QTLs are shown in bold. Suggestive QTLs with LOD score between 3.0 and the LOD threshold are shown in italics. QTLs being insignificant in a condition but significant in another are shown in grey.

<sup>b</sup> position of 2-LOD support QTL confidence interval borders

**Supplementary Table S5.** Parameters associated with the quantitative trait loci (QTLs) identified for disease resistance against scab (experiment Vi-B04\_2) and fire blight (experiment Ea-1430\_2) in the TxF progeny after water- or ASM-treatment.

<sup>a</sup> LOD thresholds were obtained after permutations (n=1000): 4.70 (Vi-B04\_water); 4.50 (Vi-B04\_ASM); 5.82 (Vi-B04<sub>qT1</sub>-\_water); 7.33 (Vi-B04<sub>qT1</sub>-\_ASM); 4.77 (Ea-1430\_water); 5.27 (Ea\_1430\_ASM). Significant QTLs are shown in bold. Suggestive QTLs with LOD score between 3.0 and the LOD threshold are shown in italics. QTLs being insignificant in a condition but significant in another are shown in grey.

<sup>b</sup> position of 2-LOD support QTL confidence interval borders

Trait	Treatment	Linkage group	Position	LOD <sup>a</sup>	R <sup>2</sup>	CI <sup>b</sup>
Vi-B04_2	water	T1	43.03	35.2	40.42	41.01 – 44.23
		T13	44.94	2.1	-	-
		F3	13.36	1.6	-	-
		F11	5.53	5.3	4.99	0.00 – 7.91
		F12	25.99	3.9	3.54	0.00 – 46.60
	F17	18.92	14.4	13.30	16.52 – 20.90	
	ASM	T1	43.03	34.5	48.1	41.01 – 45.43
		T13	0.40	1.1	-	-
		F3	22.88	2.1	-	-
		F11	5.53	2.9	-	-
F12		25.99	2.2	-	-	
F17	18.92	12.5	21.8	16.52 – 20.90		
Vi-B04_2_qT1-	water	T13	0.40	5.4	12.7	0.00 – 6.80
		F3	13.36	4.3	4.9	9.81 – 18.54
		F11	20.98	5.2	13.8	8.69 – 26.31
		F12	25.99	4.9	11.2	20.46 – 31.56
		F17	18.92	14.3	37.3	16.52 – 22.48
	ASM	T13	0.40	3.3	3.3	0.00 – 6.80
		F3	13.36	3.9	5.4	8.25 – 18.54
		F11	26.31	5.4	6.9	20.98 – 33.17
		F12	24.82	3.7	7.9	20.46 – 31.56
		F17	18.92	19.2	33.7	16.52 – 20.90
Ea-1430_2	water	T1	7.64	1.5	-	-
		T3	27.16	5.0	9.4	21.57 – 32.02
		T5	63.66	4.1	6.3	57.93 – 65.65
		T7	3.19	1.1	-	-
		T15	29.70	3.6	9.3	21.68 – 32.50
		F1	29.65	1.1	-	-
		F5	47.65	4.4	7.4	42.08 – 61.62
	ASM	F7	65.12	1.8	-	-
		F15	86.70	3.2	4.2	81.15 – 92.65
		T1	0.40	3.3	5.1	0.00 – 58.27
		T3	15.93	1.3	-	-
		T5	49.55	5.1	5.3	43.24 – 53.14
		T7	23.12	4.1	4.5	15.73 – 29.10
		T15	40.23	2.5	-	-
		F1	26.12	5.9	7.0	15.4 – 31.63
F5	47.65	3.9	9.1	42.08 – 61.62		
F7	63.13	1.6	-	-		
F15	96.57	1.1	-	-		

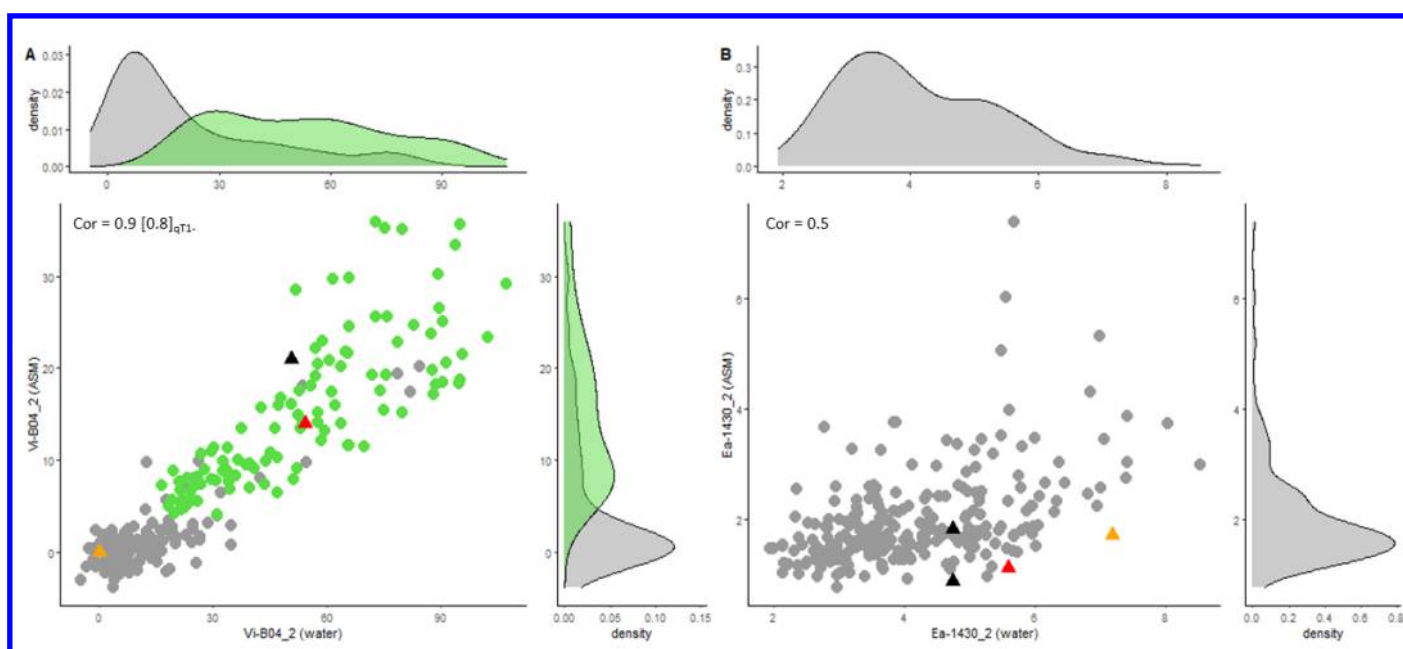


**Supplementary Table S6.** Interactions between significant and suggestive QTLs identified in Vi-B04\_2, Vi-B04\_2<sub>qT1</sub>- and Ea-1430\_2 experiments.

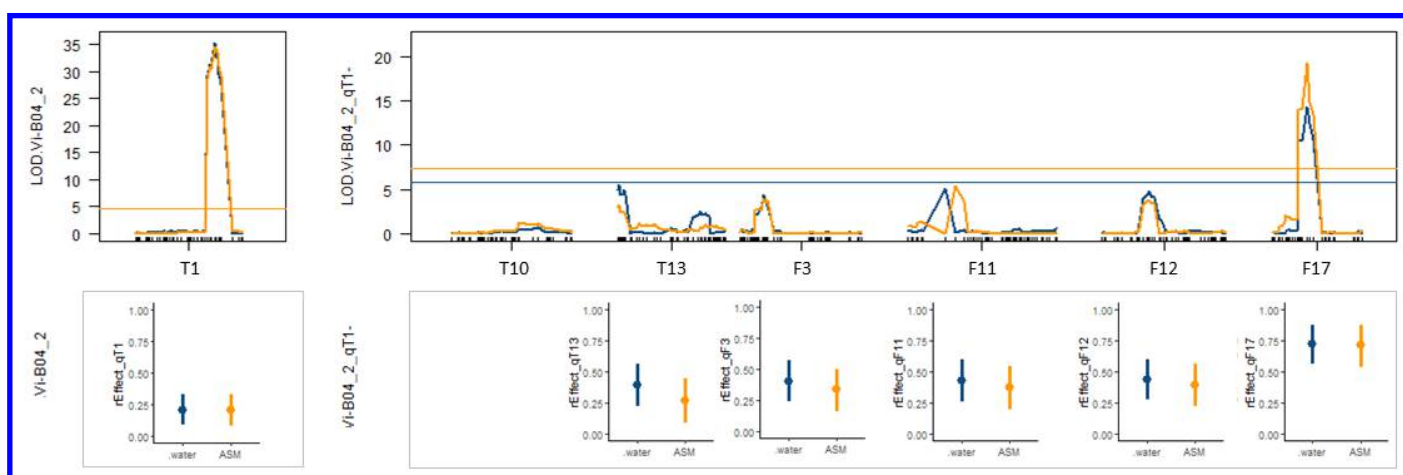
Trait	QTLs combination	water					ASM				
		AA:AA	AB:AA	AA:AB	AB:AB	F-value <sup>a</sup>	AA:AA	AB:AA	AA:AB	AB:AB	F-value <sup>a</sup>
Vi-B04_2	qF11:qF17	29.8	33.2	11.1	20.6	5.4 *	-	-	-	-	-
	qT1:qF17	-	-	-	-	-	17.0	0.5	5.9	-0.1	5.5 *
Vi-B04_2 <sub>qT1</sub>	qT13:qF11	56.3	65.1	64.6	84.3	9.0 **	-	-	-	-	-
	qT13:qF17	78.1	97.2	53.0	63.7	7.7 **	-	-	-	-	-
	qF12:qF17	90.1	83.7	65.8	47.9	5.0 *	-	-	-	-	-
Ea-1430_2	qT3:qT15	4.0	5.0	3.8	3.9	10.5 **	-	-	-	-	-
	qT5:qF5	4.0	3.7	5.0	4.1	7.0 **	-	-	-	-	-
	qT1:qF5	-	-	-	-	-	1.8	1.8	2.4	1.9	4.8 *
	qF1:qF5	-	-	-	-	-	1.9	1.8	2.4	1.8	4.0 *

<sup>a</sup> F-value of the variance analysis Fisher-test where stars represent the significance of the test at the risk of 0.05 (\*), 0.01 (\*\*) or 0.005 (\*\*\*).

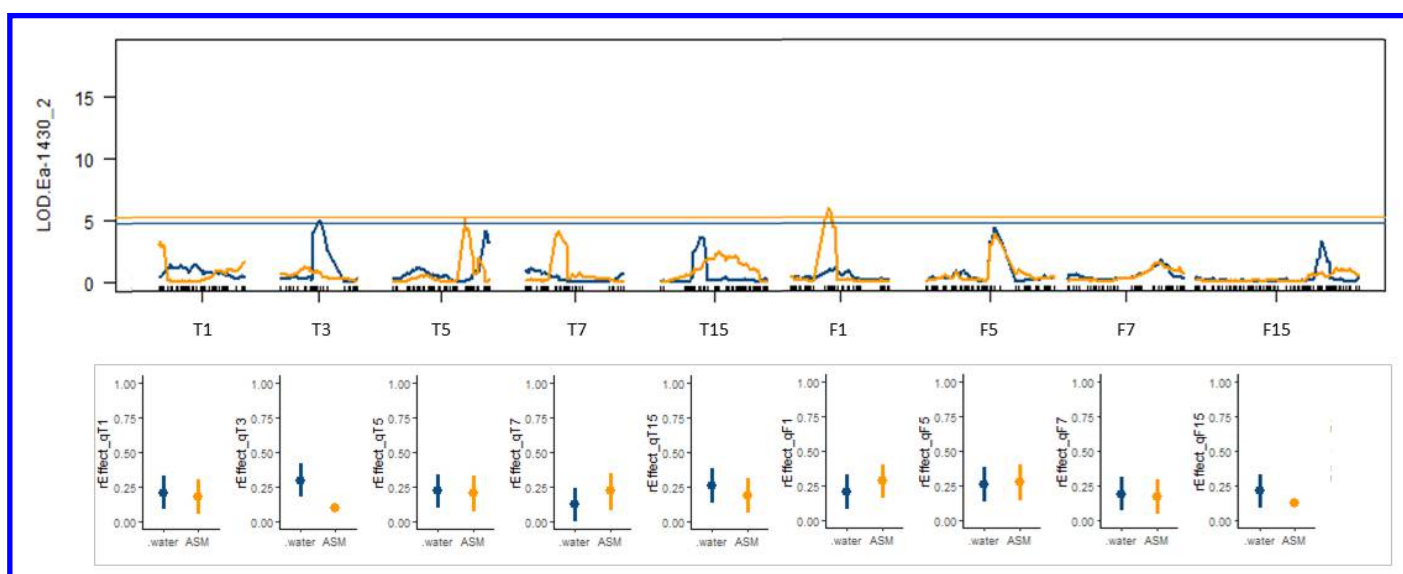
**Supplementary Figure S1.** Relationship between disease severity (BLUP estimates) for water- and ASM- treated individuals of the TxF progeny in (A) Vi-B04\_2 and (B) Ea-1430\_2 experiments. Distributions of BLUPs are shown in the upper (water control) and right (ASM treatment) part of each graph where the complete population is represented in grey and the sub-population qT1- (i.e., individuals selected as not-carrying the resistance allele of the major QTL qT1) in green. The same color code is used in the graph where the green dots are superimposed on the grey dots. Control and parental genotypes are shown with triangles in orange (TN10-8), red (Fiesta) and black (Gala and Golden Delicious). Cor is the Pearson coefficient of correlation between water and ASM BLUPs. Value for the sub-population qT1- is indicated within brackets.



**Supplementary Figure S2.** LOD score curves of QTLs involved in resistance against scab (Vi-B04\_2) for each treatment identified in the TxF progeny (upper part) and relative effects (rEffects) of these QTLs (lower part). Blue and orange colors represent respectively the water control and ASM treatment. LOD score thresholds are drawn as horizontal lines with the respective colors. In each rEffect plot, mean and standard deviation of each rEffect, calculated with bootstrapping, are represented by a point and a vertical bar respectively, with the respective blue and orange color for water-control and ASM-treatment respectively.



**Supplementary Figure S3.** LOD score curves of QTLs involved in resistance against fire blight (Ea-1430\_2) for each treatment identified in the TxF progeny (upper part) and relative effects (rEffects) of these QTLs (lower part). Blue and orange colors represent respectively the water control and ASM treatment. LOD score threshold are drawn as horizontal lines with the respective colors. In each rEffect plot, mean and standard deviation of each rEffect, calculated with bootstrapping, are represented by a point and a vertical bar respectively, with the respective blue and orange color for water-control and ASM-treatment respectively.



**Supplementary Table S3.** Summary of the genetic map of the TxF progeny. For each linkage group of each parent, SNP markers are ordered according to their genetic position given in centimorgan (cM).