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Abstract

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

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

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

Introduction

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

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

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

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

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

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

Materials and Methods

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

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

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

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

described in Calenge et al. (2004). Two experiments were performed with isolate EU-B04 (coded Vi-B04_1 et Vi-B04_2) and one with 09BCZ014 (coded Vi-Z14).

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

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

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

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

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

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

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

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

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

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

Results

Phenotypic variability. Only experiments involving the most replicates per individual, Vi-B04_1, Vi-Z14 and Ea-1430_1 are presented here (the two additional experiments Vi-B04_2 and Ea-1430_2 are available as supplementary data).

A significant reduction of AUDPC was observed for both scab and fire blight after ASM treatment compared to the water control (Table 1). For the TxF progeny, AUDPC was reduced by 54% for Vi-B04_1, 73% for Vi-Z14 and 50% for Ea-1430_1. Similar reductions were observed for the two parents and susceptible controls (Figure 1). A large variation was observed among individuals regardless of the conditions, with AUDPC values ranging from 0 to 150 for Vi-B04_1, from 0 to 75 for Vi-Z14 and from 1 to 11 for Ea-1430_1 (Figure 1). A higher coefficient of variation was observed for scab (CV=0.75 – 1.27) than for fire blight (CV=0.27 – 0.34; Table 1). The distributions were generally unimodal except for the water control in Vi-B04_1 where a clear bimodal distribution was observed (Figure 1A), fitting with the expected effect of QTL qT1 (see Materials and Methods). Broad-sense heritabilities (h^2) were generally high whatever the conditions, ranging from 0.63 for fire blight after ASM treatment to 0.94 for scab after the water control (Table 1). Adjusting the data for spatial effects resulted in a limited increase of heritability from 0.01 to 0.08 units compared to no adjustment (data 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

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

For experiment Vi-B04_2, the same QTLs were detected despite at lower LOD scores than in the first experimentation (Supplementary Table S5). In the whole TxF progeny, qT1 was still detected as the strongest QTL for both treatment conditions (LOD ~ 35; R^2 ~40-48%) together with qF11 and qF12 as suggestive QTL (water control) and qF17 (both conditions) and still a significant interaction between qF11 and qF17 (Supplementary Table S6, water control). In the qT1- sub-population, qF17 was the single significant QTL detected, while qT13, qF3, qF11 and qF12 were detected as suggestive QTLs with mostly similar R^2 compared to the Vi-B04_1 experiment. Three significant interactions between QTLs were detected, two of them involving qT13 with qF11 and qF17 respectively (Supplementary Table S6). In this experiment, ASM treatment did not significantly modulate the QTL effect on any of the QTLs detected (Supplementary Figure S2).

QTL detection for *V. inaequalis* isolate '09BCZ14'. For the water control condition, all the QTLs detected in Vi-B04_1 were also detected in Vi-Z14, except on LG F12, and a new suggestive QTL was detected on LG T10. The phenotypic variation explained by these QTLs ranged from 3.5 to 10.7% and the relative effects from 0.25 (QTL qT10) to 0.42 (QTLs qT13 and qF17) (Table 2, Figure 2). Interaction between QTLs qF11 and qF17 was still significant whereas new interactions were found significant between qT1 and qF3, between qT13 and qF3 and between qT10 and qF11 (Table 3). Altogether, these QTLs and interactions explained 47.5% of the phenotypic variation (Table 1). For ASM-treatment condition, three out of the six QTLs identified in the water control were still detected but only as suggestive QTLs (qT13, qF11, qF17; Table 2). Altogether, they explained only 15.0% of the phenotypic variation without any interactions. The relative effects of these QTLs were not significantly different when

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

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

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

Discussion

A first result of our study has been to confirm already known scab and fire blight 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.

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

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

Vi-B04_1, and qT1 for Vi-Z14) almost disappeared after treatment with ASM. We also detected two fire blight resistance QTLs (qT3, qF15) which effects significantly decreased after ASM treatment in the second Ea-1430 experiment, but the experimental conditions were questionable. Despite a large mapping population, the confidence intervals surrounding relative effects of QTLs were large and therefore precluded the detection of low modulation of QTL effects by ASM. More individuals and replicates per individual would have been necessary to detect such modulations. Furthermore, no QTL specific to the ASM treatment condition was detected: all QTLs detected with ASM were already identified as resistance QTLs under the water control. The general trend was either a maintenance or a reduction in the relative effect of QTLs. Some of them fully disappeared (significantly) but this observation was hard to repeat for both diseases. To some extent, this QTL effect disappearance could also be explained by a scale effect. Indeed, the application of ASM strongly reduces the global variability in disease severity and the weakest QTLs under the water control can become insignificant under ASM treatment due to a consequent lack of statistical power. However, a general trend of maintenance or small reduction of most QTLs is surprising: considering the overall effectiveness of ASM on reducing disease symptoms and the interactions observed between less resistant genotypes and treatment, it would have been expected to found more interactions between pathways leading to this resistance.

To go further, according to the diverse nature of the QTLs present in our population, we expected to observe some QTLs responding to ASM and others remaining unchanged. ASM is known to act on salicylic acid signaling pathway. In tobacco, it is perceived by the SABP2 protein (Salicylic acid-binding protein 2) which transforms it 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.

Our study revealed that the intrinsic resistance controlled by QTLs and the resistance induced by ASM have a complementary effect for the control of apple scab and fire blight. In our study, the average effectiveness of ASM in reducing the disease ranged from 50% for Ea-1430_1 to 78% for V1-B04_2. Under the water control, the individuals cumulating favorable alleles at all detected QTLs exhibited a disease reduction of 91% for Vi-B04_1 and 98% for Vi-Z14 compared to individuals carrying only unfavorable QTL alleles for scab, and 78% for fire blight. When intrinsic and ASM-induced resistance was combined, the disease reduction was close to 100% for scab and 85% for fire blight. These results confirm that there is no incompatibility between intrinsic and ASM-induced resistance for apple as reported for cucumber (da Rocha and Hammerschmidt, 2005). The present results were obtained under controlled conditions but arguments suggest that the same trends could be observed in orchards. On the one hand, Caffier et al. (2014, 2016) showed that quantitative resistance related to QTLs qT1, qF11 and qF17 significantly reduced scab severity in orchards. On the other hand, Marolleau et al. (2017) confirmed that ASM could be integrated into orchard protection practices to control apple scab. Combining both types of resistance in the orchard should allow a better control of apple scab and similarly for fire blight.

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

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

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Tables

Table 1. Mean values (± 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* isolates EU-B04 and 09BCZ14; experiments Vi-B04_1 and Vi-Z14, respectively) and fire blight (*E. amylovora* isolate CFPB1430; experiment Ea-1430_1) 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_1	water	41.2 ± 36.8	54	0.84 [0.29]$_{qT1-}$	0.94	81.9 [65.1]$_{qT1-}$
	ASM	18.9 ± 26.4		1.27 [0.48]$_{qT1-}$	0.91	70.2 [53.8]$_{qT1-}$
Vi-Z14	water	24.1 ± 21.4	73	0.75	0.84	47.5
	ASM	6.6 ± 8.4		0.88	0.71	15.0
Ea-1430_1	water	6.2 ± 2.4	50	0.27	0.70	35.0
	ASM	3.1 ± 1.8		0.34	0.63	33.1

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 [a] LOD thresholds were obtained after permutations (n=1000): 5.1 (Vi-B04$_{qT1\text{-}}$_water), 4.6 (Vi-B04$_{qT1\text{-}}$_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 [b] position of 2-LOD support QTL confidence interval borders

768

Trait	Treatment	Linkage group	Position	LOD [a]	R^2	CI [b]
Vi-B04_1$_{qT1-}$	water	**T13**	**3.58**	**8.6**	**9.3**	**0 .00 – 6.80**
		F3	**13.36**	**5.7**	**5.5**	**8.25 – 18.54**
		F11	**5.00**	**10.7**	**18.1**	**0.00 – 20.98**
		F12	*25.99*	*3.0*	*3.7*	*20.46 – 31.56*
		F17	**18.92**	**12.4**	**29.0**	**16.52 – 27.35**
	ASM	T13	3.58	0.3	-	-
		F3	44.80	0.8	-	-
		F11	**0.00**	**7.6**	**10.7**	**0.00 – 7.91**
		F12	*19.28*	*4.0*	*5.1*	*10.83 – 24.43*
		F17	**18.00**	**21.2**	**39.9**	**16.52 – 20.90**
Vi-Z14	water	**T1**	**45.03**	**9.4**	**10.7**	**39.01 – 52.89**
		T10	*62.09*	*3.6*	*3.5*	54.88 – 65.69
		T13	**3.18**	**10.9**	**10.1**	**0.00 – 6.80**
		F3	**3.92**	**5.1**	**5.3**	**0.00 – 9.03**
		F11	**5.53**	**7.8**	**7.9**	**0.00 – 20.98**
		F17	**18.92**	**9.1**	**10.7**	**14.18 – 27.35**
	ASM	T1	36.61	1.7	-	-
		T10	62.09	2.2	-	-
		T13	*0.00*	*4.1*	*6.3*	*0.00 – 6.80*
		F3	5.50	1.1	-	-
		F11	*3.17*	*3.9*	*5.0*	*0.00 – 8.69*
		F17	*18.92*	*4.2*	*5.0*	*13.79 – 27.35*
Ea-1430_1	water	*T3*	*23.57*	*4.1*	*3.1*	*11.81 – 26.76*
		T5	**54.33**	**5.8**	**5.2**	**43.24 – 62.05**
		F5	39.73	2.5	-	-
		F7	**69.46**	**18.4**	**20.1**	**67.10 – 71.42**
		F15	*76.05*	*3.7*	*3.9*	*70.94 – 81.15*
	ASM	*T3*	*20.38*	*3.7*	*4.6*	*17.13 – 29.18*
		T5	**35.22**	**4.9**	**4.6**	**28.37 – 40.84**
		F5	**38.56**	**6.3**	**8.4**	**32.18 – 42.08**
		F7	**69.07**	**12.2**	**13.2**	**67.10 – 71.42**
		F15	86.70	2.9	-	-

769

770 **Table 3**. Interactions between significant and suggestive QTLs identified in Vi-B04_1$_{qT1.}$ and Vi-Z14 experiments.

771

		water					ASM				
Trait	**QTLs combination**	**AA:AA**	**AB:AA**	**AA:AB**	**AB:AB**	**F-value [a]**	**AA:AA**	**AB:AA**	**AA:AB**	**AB:AB**	**F-value [a]**
Vi-B04_1$_{qT1.}$	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 [a] 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 (***).

Figures Captions

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 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.

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 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

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

801 and ASM-treatment respectively.

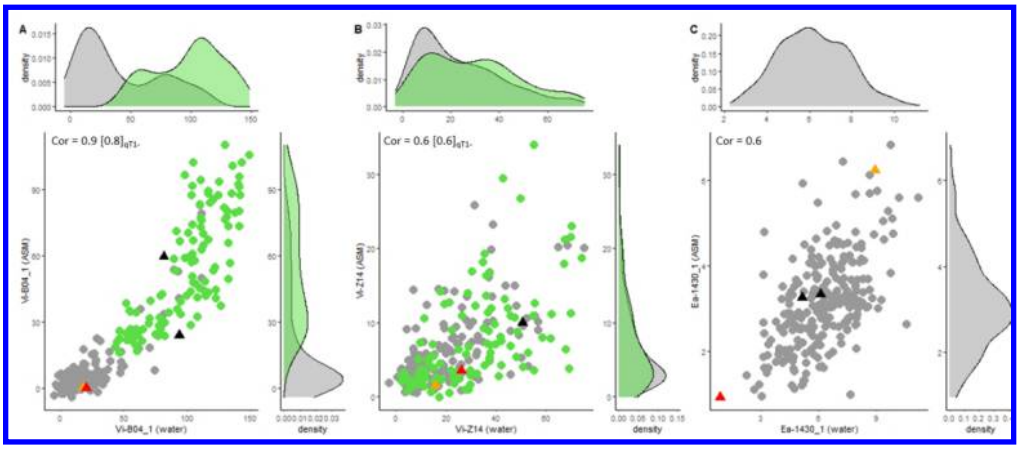


Figure 1. Relationship between disease severity (BLUP estimates) for water- and ASM-treated individuals of the TxF progeny in (A) Vi-B04_1, (B) Vi-Z14 and (C) Ea-1430 experiments. Distributions of BLUPs are shown in the upper (water control) and right (ASM treatment) part of each graph where the complete 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.

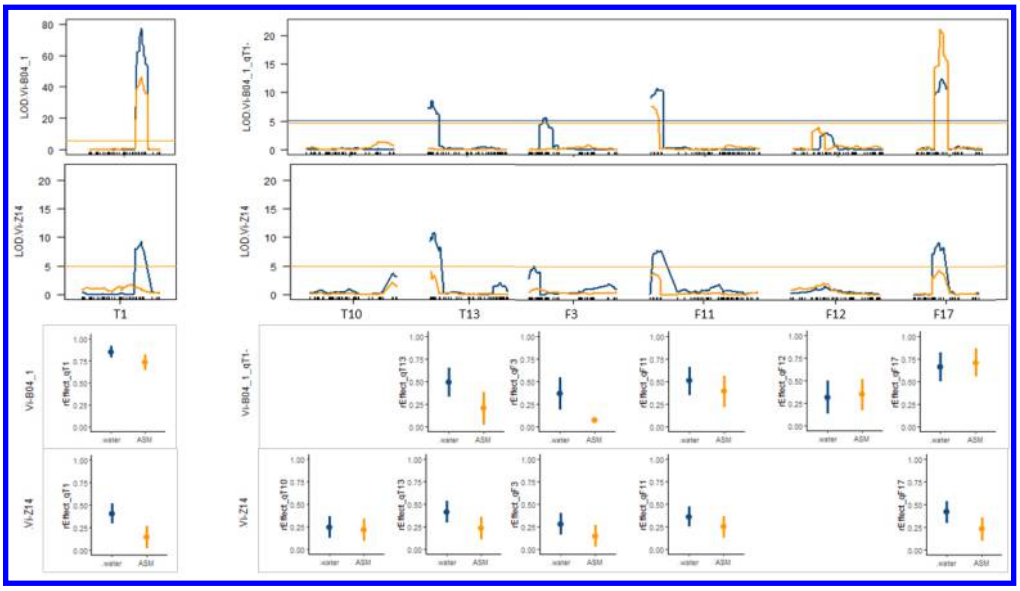


Figure 2. LOD score curves of QTLs involved in resistance against scab (Vi-B04_1 and Vi-Z14) for each treatment identified in the TxF progeny (upper part) and relative effects (rEffects) of these QTLs (lower part). Blue and orange colors represent respectively water control and ASM treatment. LOD score 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.

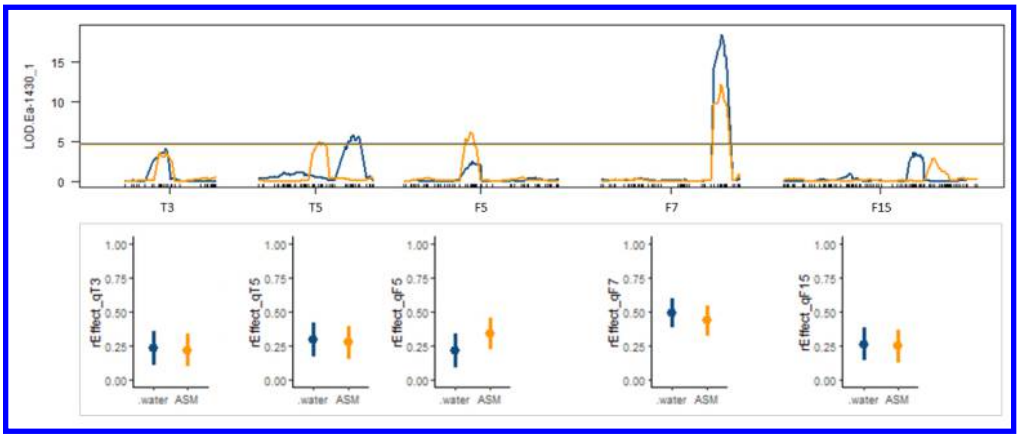


Figure 3. LOD score curves of QTLs involved in resistance against fire blight (Ea-1430_1) for each treatment identified in the TxF progeny (upper part) and relative effects (rEffects) of these QTLs (lower part). Blue and orange colors represent respectively water control and ASM treatment. LOD score 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 (± 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 ± 26.2		1.03 [0.47]$_{qT1-}$	0.89	56.1 [68.8]$_{qT1-}$
	ASM	5.1 ± 8.9	78	1.62 [0.59]$_{qT1-}$	0.85	56.3 [56.7]$_{qT1-}$
Ea-1430_2	water	4.2 ± 2.5		0.30	0.51	30.2
	ASM	2 ± 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 [a]	R^2	CI [b]
Vi-B04_1	water	**T1**	**43.00**	**77.1**	**69.9**	**41.01 – 44.23**
		T13	**3.58**	**6.3**	**2.8**	**0.00 – 6.80**
		F3	12.19	1.6	-	-
		F11	**6.00**	**8.9**	**7.7**	**0.00 – 20.98**
		F12	*4.00*	*3.3*	*0.6*	*0.00 – 6.87*
		F17	**18.92**	**16.7**	**12.8**	**16.52 – 20.90**
	ASM	**T1**	**43.03**	**46.1**	**60.0**	**41.01 – 44.23**
		T13	3.58	0.8	-	-
		F3	1.96	0.3	-	-
		F11	*4.75*	*4.3*	*6.9*	*0.00 – 20.98*
		F12	19.28	2.3	-	-
		F17	**18.92**	**15.9**	**24.9**	**16.52 – 20.90**

[a] 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.

[b] 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.

[a] LOD thresholds were obtained after permutations (n=1000): 4.70 (Vi-B04_water); 4.50 (Vi-B04_ASM); 5.82 (Vi-B04$_{qT1}$-_water); 7.33 (Vi-B04$_{qT1}$-_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.

[b] position of 2-LOD support QTL confidence interval borders

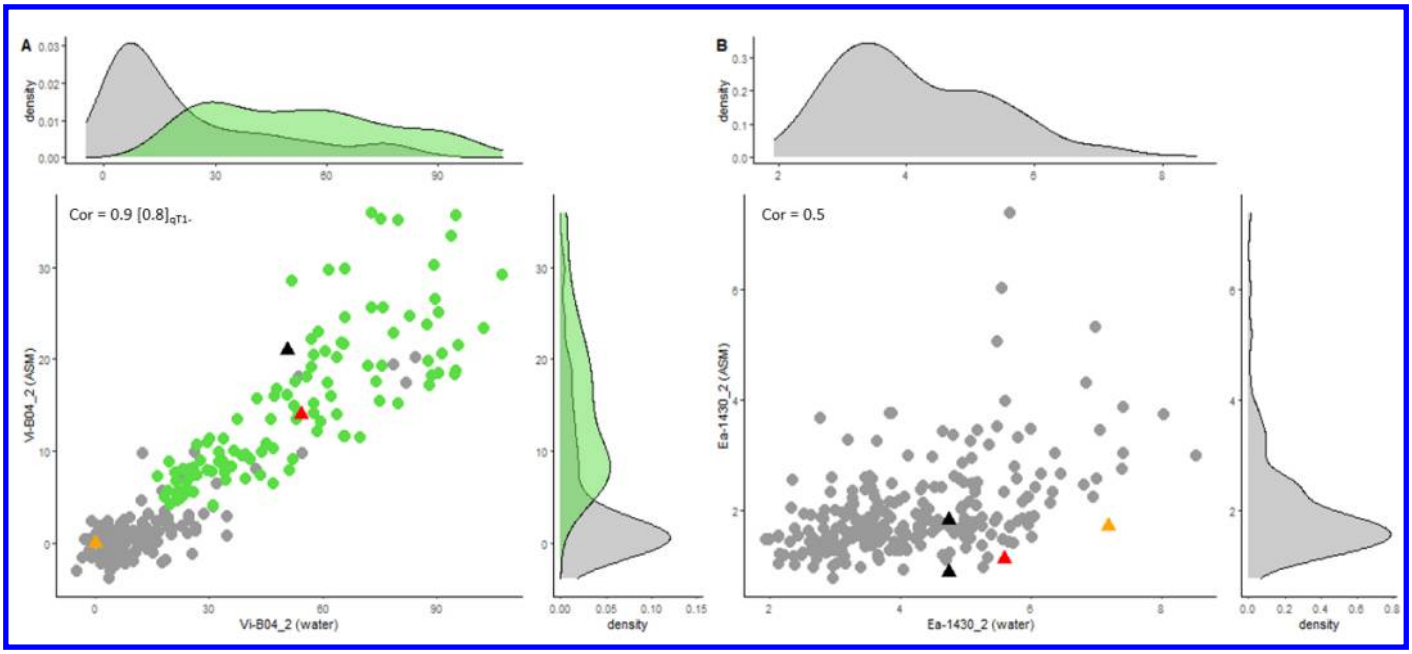
Trait	Treatment	Linkage group	Position	LOD [a]	R^2	CI [b]
Vi-B04_2	water	**T1**	**43.03**	**35.2**	**40.42**	**41.01 – 44.23**
		T13	44.94	2.1	-	-
		F3	13.36	1.6	-	-
		F11	**5.53**	**5.3**	**4.99**	**0.00 – 7.91**
		F12	*25.99*	*3.9*	*3.54*	*0.00 – 46.60*
		F17	**18.92**	**14.4**	**13.30**	**16.52 – 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
		F7	65.12	1.8	-	-
		F15	*86.70*	*3.2*	*4.2*	*81.15 – 92.65*
	ASM	*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$_{qT1-}$ and Ea-1430_2 experiments.

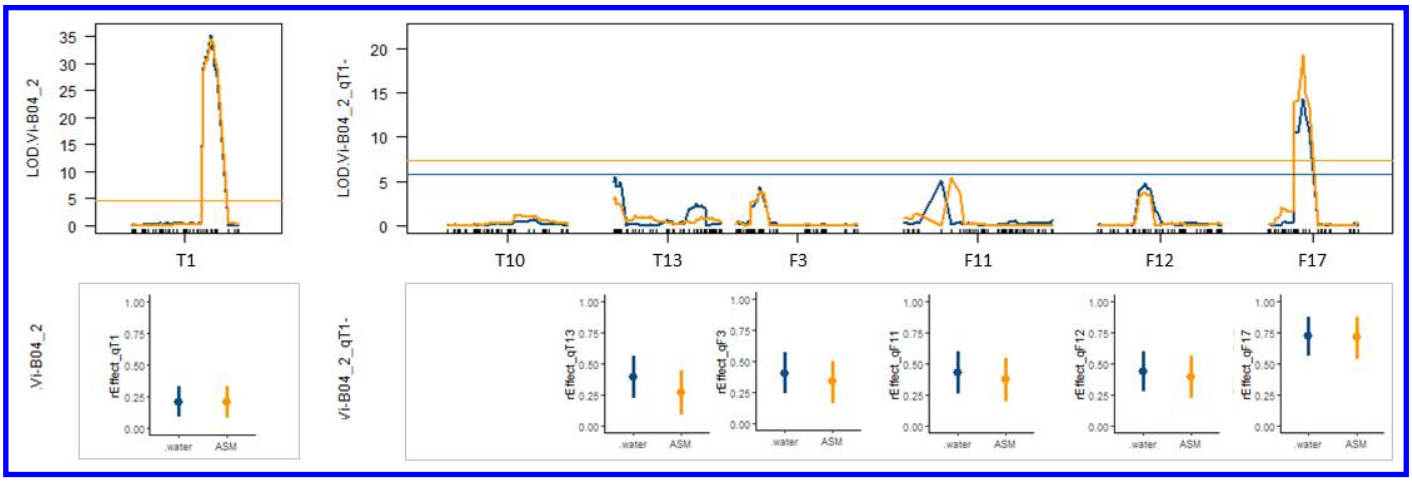
		water					ASM				
Trait	**QTLs combination**	**AA:AA**	**AB:AA**	**AA:AB**	**AB:AB**	**F-value [a]**	**AA:AA**	**AB:AA**	**AA:AB**	**AB:AB**	**F-value [a]**
Vi-B04_2	qF11:qF17	29.8	33.2	11.1	20.6	5.4 *	-	-	-	-	-
	qT1:qF17	-	-	-	-	-	17.0	0.5	5.9	-0.1	5.5 *
Vi-B04_2$_{qT1-}$	qT13:qF11	56.3	65.1	64.6	84.3	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 *

[a] 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 (***).

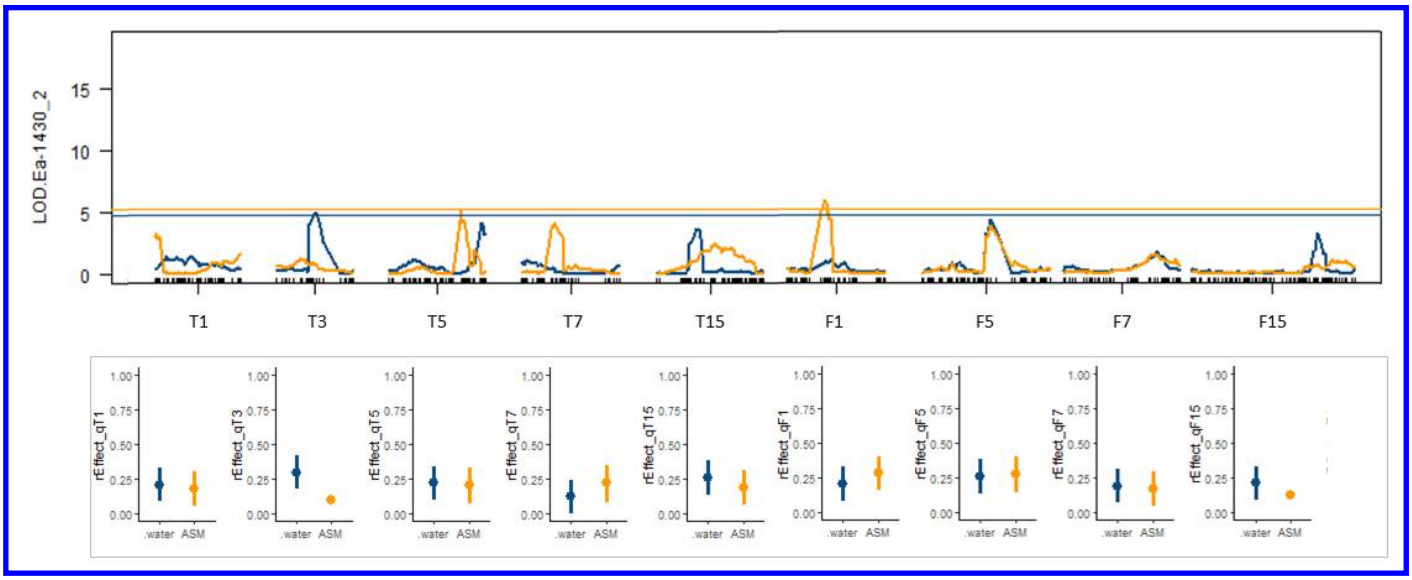
Supplementary Figure S1. Relationship between disease severity (BLUP estimates) for water- and ASM- treated individuals of the TxF progeny in (A) Vi-B04_2 and (B) Ea-1430_2 experiments. Distributions of BLUPs are shown in the upper (water control) and right (ASM treatment) part of each graph where the 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).