Tree Genetics & Genomes (2019) 15:25
https://doi.org/10.1007/s11295-019-1332-y

ORIGINAL ARTICLE

Genetic mapping of the European canker (*Neonectria ditissima*) resistance locus *Rnd1* from *Malus* 'Robusta 5'

Vincent G. M. Bus[1] · Reiny W. A. Scheper[1] · Monika Walter[2] · Rebecca E. Campbell[2] · Biff Kitson[2] · Lauren Turner[2] · Brent M. Fisher[1] · Sarah L. Johnston[1] · Chen Wu[3] · Cecilia H. Deng[3] · Gagandeep Singla[1] · Deepa Bowatte[4] · Linley K. Jesson[1] · Duncan I. Hedderley[4] · Richard K. Volz[1] · David Chagné[4] · Susan E. Gardiner[4]

Received: 9 December 2018 / Revised: 13 February 2019 / Accepted: 4 March 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Current control strategies for the major apple disease European canker (EC) are laborious and expensive, and often do not prevent progression of the disease, which can lead to loss of trees and therefore production. Hence, the development of resistant cultivars is a significant goal for breeders supporting growers in maritime climates conducive to the disease. With genetic markers increasingly being used as a tool in marker-assisted selection for parental and seedling selection, genetic mapping of major effect loci controlling resistance to the pathogen is integral to most breeding programmes. We report the genetic mapping of EC resistance in a bi-parental progeny derived from a cross between moderately EC-resistant 'Malling 9' ('M9') and highly resistant *Malus* 'Robusta 5' (R5) using two resistance phenotyping techniques. Field inoculation of rasp wounds on the stem and lateral shoots of replicated plants grown on their own roots with a suspension of *Neonectria ditissima* conidia proved both easier to perform and more effective than inoculation onto leaf scars. Rasp wound phenotype data combined with a previously reported genetic map enabled us to identify a large-effect QTL for control of resistance to EC on linkage group 14 of R5, which we named *Rnd1*. The position of this QTL was confirmed using leaf scar phenotyping data from the field and glasshouse inoculations. We have developed new SNP markers for this locus, using a novel bioinformatic SNP filtering tool that searches aligned genomic sequences of multiple apple accessions. We have converted one of these markers into a high-throughput version for application in marker-assisted selection of apple.

Keywords Apple · Leaf scar · Rasp wound · QTL · High-throughput marker · Marker-assisted selection

Introduction

European canker (EC) (*Neonectria ditissima*; syn. *Nectria galligena*) is a major intractable woody tissue disease of apple grown under maritime climates, i.e. growing conditions with moderate temperatures and a fair amount of precipitation (Weber 2014). The disease affects the woody parts of the tree and can readily kill part of, or even the whole tree by ringbarking of branches and tree stems, or by killing the root-stock. In the northern hemisphere, the pathogen has also been reported to affect fruit, causing 'eye rot', which can contribute to postharvest losses (Grove 1990). Disease control primarily relies on the persistent and thorough removal of infected shoots and branches (Weber 2014), while preventive strategies include using pruning paints and fungicide applications to protect pruning cuts, leaf scars and picking wounds, the major entry points for infection under New Zealand production

Communicated by V. Decroocq

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11295-019-1332-y) contains supplementary material, which is available to authorized users.

✉ Vincent G. M. Bus
Vincent.Bus@plantandfood.co.nz

[1] The New Zealand Institute for Plant and Food Research Ltd (Plant & Food Research), Hawke's Bay Research Centre, Havelock North, New Zealand

[2] Plant & Food Research, Motueka Research Centre, Motueka, New Zealand

[3] Plant & Food Research, Mt Albert Research Centre, Auckland, New Zealand

[4] Plant & Food Research, Palmerston North Research Centre, Palmerston North, New Zealand

Published online: 13 March 2019

conditions (Amponsah et al. 2015; Campbell et al. 2016). However, EC removal and painting of pruning cuts is laborious and therefore expensive, and preventative control strategies based on agrichemicals typically only slow, but not prevent, disease progression (Cooke 1999).

In the long term, breeding genetically resistant, or ideally immune, cultivars provides a lasting solution, provided the resistance is durable, which can be achieved by pyramiding different resistances into the same cultivar (Bus et al. 2009). Early research in the UK identified 'Bramley's Seedling', 'Grenadier', 'Early Victoria', 'Gladstone', 'Lane's Prince Albert' and 'Newton Wonder' as the more resistant scion cultivars (Bagenal 1945; Wormald 1955). Of the apple rootstocks, 'M1' and 'M12' were found to be highly resistant, while 'M16', 'M4', 'M7', 'M2' and 'M9' were moderately resistant (Moore 1960). More recently, Gómez-Cortecero et al. (2016) and Scheper et al. (2017) reported the relatively low susceptibility of 'M116' and 'MM106', and confirmed that 'M9' is moderately resistant, although clonal selection 'M9'-T337 was found to be highly infected in one experiment (Gómez-Cortecero et al. 2016). Rootstocks were also found to affect the susceptibility of scion cultivars (Moore 1934; Umpleby and Swarbrick 1936; Scheper et al. 2017). In a replicated study, Scheper et al. (2017) found that resistant rootstocks from an 'M9' × 'Robusta 5' (M9×R5) family decreased susceptibility of the susceptible 'Royal Gala' scion, but did not affect susceptibility of the moderately resistant 'Golden Delicious'.

Further germplasm evaluations have confirmed previous findings and/or identified additional (moderately) resistant accessions, for example, 'Dunkitt', 'Early Victoria', 'Gladstone', 'Greasy Pippin' (Alston 1970), 'Beauty of Rept', 'Boiken', 'Close', 'Wealthy' (Zagaja et al. 1971), 'Astramel', Klon 40, 'Cortklar' (Krüger 1983), 'Jonathan', 'Lombart's Calville' (Van de Weg 1989), 'Prairifire', 'Kim', 'Santana' (Garkava-Gustavsson et al. 2013), 'Bananovoye', 'Imant', 'Minskoye', 'Fameuse', 'Peppin Litowskii', 'Sinap Belsad' (Kazlouskaya and Marchuk 2013) and 'Golden Delicious' (Garkava-Gustavsson et al. 2016; Gómez-Cortecero et al. 2016; Van de Weg 1989). Sometimes the studies showed contrasting findings, which may be attributed to different phenotyping methods having been used. For example, 'Worcester Pearmain' has been reported as resistant in a glasshouse screen (Garkava-Gustavsson et al. 2013) and susceptible in general field observations (Wormald 1955), while 'Elstar' was resistant in an excised shoot study (Van de Weg 1989), but moderately susceptible under field conditions (Palm et al. 2011). The choice of phenotyping method will affect the resistance component that is being targeted, such as disease incidence and lesion growth rates, which may be under different genetic controls (Wenneker et al. 2017). Variations in experimental and environmental conditions further complicate resistance phenotyping (Beresford and Kim 2011; Dubin and English 1974; Garkava-Gustavsson et al. 2013, 2016; Ghasemkhani et al. 2015b; Gómez-Cortecero et al. 2016; Xu et al. 1998). Resistance comparisons can be further improved by ensuring that wood of similar age is used, since disease expression has been reported to be greater on older wood (Amponsah et al. 2017) and on new (green) shoot growth when compared with younger wood (Børve et al. 2017). For wounds on the same shoot, the ones positioned towards the top tend to express more severe symptoms than those at the lower end (Wenneker et al. 2017).

Preliminary genetic studies indicated that resistance to EC was quantitatively inherited, with additive gene action (Gelvonauskiene et al. 2007; Gómez-Cortecero et al. 2016; Krüger 1983), and that some cultivars conferred susceptibility rather than resistance to the disease. In this study, we present genetic analysis of the EC resistance of crab apple *Malus* 'Robusta 5' (R5). This accession is highly resistant (Bus et al. 2017; Gómez-Cortecero et al. 2016; Scheper et al. 2017), with preliminary genetic studies reporting a high proportion of resistant progeny, suggesting that the resistance is sufficiently heritable for use in the genetic improvement of apple cultivars. Being moderately resistant, the rootstock 'M9' might also have useful quantitative trait loci (QTLs) to contribute (Gómez-Cortecero et al. 2016). Our genetic mapping was performed in the same M9×R5 family used for the mapping of two loci in 'M9' controlling scion dwarfing (Foster et al. 2015; Rusholme Pilcher et al. 2008), as well as the woolly apple aphid (Bus et al. 2008) and fire blight (Gardiner et al. 2012; Peil et al. 2008) resistance loci from R5, *Er2* and *FBR5*, respectively.

Materials and methods

Plant material

The M9×R5 family comprising 159 progeny (Celton et al. 2009) is maintained as stoolbeds at the Plant & Food Research (PFR) orchard in Havelock North (Hawke's Bay, New Zealand), where each stoolbed is identified by a leaf sample number starting with AJ. Phenotyping of individual plants for resistance to *N. ditissima* was performed in the glasshouse (2012, 2014) as well as almost the whole population in the field (2016). The glasshouse evaluation involved randomly selected sub-sets of 44 and 24 plants in 2012 and 2014, respectively.

Glasshouse phenotyping

In August 2011 and 2013, own-rooted trees of 36 and 22 progeny, respectively, and the 'M9' parent were harvested from the stoolbeds and potted into 3.5-L plastic planter bags

in Havelock North. The plants were maintained on a hardstand until April 2012 and 2014, when they were brought inside a glasshouse in preparation for inoculation. In 2012, trees of the R5 and 'M9' parents and the reference cultivars 'Royal Gala' and 'Monty's Surprise', all grafted onto 'M9', as well as own-rooted 'M9' were included. In 2014, R5 on 'M9', own-rooted 'M9' and 'Royal Gala' on 'MM106' were included, plus ten of the progeny that had been tested in 2012. One to five non-inoculated trees of each accession were placed randomly between the inoculated trees as negative controls (not included in the analysis).

In 2012, potted 1-year-old trees were arranged in a randomised block design, with seven to ten two-tree replicates, each comprising five to eight leaf scars distributed over the two trees per accession. Leaf scars were used as infection sites because they are a natural way for the tree to become infected: they were created on the main leader by gently removing the leaves at the abscission layer. The scars were at least 15 cm apart and were marked with a white paint pen (Supplementary Fig. 1A). In 2014, potted 1-year-old trees were arranged in four to five replicates per accession, with each replicate comprising one to four trees with nine scars distributed among them. Because of advanced leaf fall, leaf scars were complemented by bud scars in those accessions for which an insufficient number of leaves were available. Bud scars were made by breaking off the buds. Only leaves and buds on the main leader were used.

During 18–22 June 2012, leaf scars were inoculated within 4 h of being made by placing 10 μL suspension of field-produced conidia suspended in 0.005% Tween®20 (1.5×10^5 conidia mL^{-1}) using a pipette (Scheper et al. 2015). The conidial suspension was prepared 1 or 2 days before inoculation from sporulating cankers collected from an orchard block in Motueka, Tasman, New Zealand, in May 2012 and stored at 1 °C for 16 or 40 h according to the method of Scheper et al. (2016). Each day, two or three replicate progeny sets were inoculated. Concurrently, the conidial germination rate of the inocula was observed after 24-h incubation on glass slides (60 μL droplets) in a humidity chamber on the laboratory bench as described by Scheper et al. (2016). With germination rates ranging between 68 and 87%, the inoculum proved to be highly viable. In 2014 using the same protocol as in 2012, leaf and bud scars were inoculated on 16 May, using field-produced conidia in sterile distilled water (2×10^5 conidia mL^{-1}). The conidial suspension was prepared on the day of inoculation and concurrent assessment showed that the germination rate after 24 h was 75%. Following inoculation, the relative humidity in the glasshouse was increased to 90% for 3 weeks, after which the humidity was kept at 80%.

The potted trees were assessed twice for the presence of lesions and lesion length was measured 15 and 25 weeks after inoculation (WAI) in October and December 2012, respectively. Disease development over time was more closely observed in three replicates by measuring incidence and lesion length nine times at 8, 10, 12, 15, 17, 19, 21, 25 and 29 WAI. Trees in the smaller trial were assessed twice, 22 and 38 WAI in October 2014 and February 2015, respectively.

Field phenotyping

Field phenotyping was performed on 122 M9×R5 progeny, 'M9' and R5 grafted onto 'M9', as well as trees of reference cultivars 'Jonathan', 'Golden Delicious' and 'Royal Gala' on 'M9' rootstock. The own-rooted trees harvested from the stoolbeds arrived at the Motueka research orchard of PFR on 24 September 2015, where the rooted parts were folded into fresh, wet sawdust and kept indoors until planting on 5 October 2015. Two to eight pairs of trees (the majority were six to seven pairs of trees) of each progeny and accessions were planted at 30 cm × 30 cm planting distance in a randomised planting design. Approximately 25–30% of the cuttings had no roots, so were dipped in a rooting hormone slurry at the manufacturer's recommended rate (Yates Clonex Rooting Hormone Hardwood Red®, Mitre 10). On 15 November 2015, plants were cut back to one or two single leaders and all cuts were covered with commercial pruning paint (Bacseal® Super, Bayer Crop Science New Zealand). The plants were grown for one season and were inoculated twice during leaf fall, on 17 and 25 May 2016—day 1 and day 2, respectively. On one of the pairwise planted trees of each progeny, leaf scars (still carrying the senescing leaves) were marked with a paint pen prior to inoculation (Walter et al. 2015). On the other tree, rasp wounds approximately 5–7 mm wide and up to 1 mm deep (Supplementary Fig. 1B,C) were made on the day of inoculation on the stems and shoots between leaf nodes (Walter et al. 2016). On inoculation day 1, three fresh leaf scars (made by removing the leaf) and three rasp wounds were brush-inoculated with about 20–30 μL, prepared as described below, on the central leaders: where available, three lateral shoots with either one rasp wound or three leaf scar inoculations each were also inoculated. On inoculation day 2, on the same plants, three more rasp wounds or six more leaf scars were made on the central leader where lateral shoots were absent, or a single rasp wound or three leaf scars were made on an additional three lateral shoots, if present. Generally, there were three inoculation sites on a central leader and six lateral shoots inoculated per main leader, with one to two main leaders/plant.

The inoculum was a mix (1:1) of frozen *N. ditissima* conidia (− 20 °C, prepared on 2 May 2016) and fresh conidia prepared on the day of inoculation from sporulating field lesions harvested locally from a Motueka orchard (Walter et al. 2015). The final spore concentration was 1×10^4 conidia mL^{-1}, with germination rates on glass slides (Walter et al. 2015) of 91% and 35% for inoculation on day 1 and day 2, respectively.

Visual assessments for EC symptoms were made on 5 September (15 WAI), 2 October (19 WAI) and 3 December 2016 (28 WAI), and 19 January 2017 (35 WAI). To avoid increasing disease pressure in the orchard, lesions were removed before they started to sporulate, i.e. the central leader or lateral branches were severed below the lesion if one or more lesions from the artificially inoculated rasp or leaf scar wounds were visible. Removing lesions closer to the stem meant symptomless wounds further up the same shoot were removed, too. Hence, at each assessment time, the number of inoculated wounds with symptoms and the number of wounds without symptoms (casualties) were recorded for each shoot that was removed. Generally, the EC lesions were small (< 10 mm) at the time of assessment. Branches and lesions were removed from the site for disposal and pruning cuts were sealed with Bacseal Super pruning paint.

Data analysis

For the glasshouse trials, the proportion of inoculated scars with lesions (disease incidence) was analysed using a binomial generalised linear model. The average lesion length for each replicate of each accession was log transformed to stabilise variance and then analysed using unbalanced ANOVA/regression. Statistical analyses were carried out using GenStat (version 14, 2011, VSNi Ltd., Hemel Hempstead, UK). In 2014, the proportion of leaf (vs bud) scars was included as a continuous variable; its effect was tested both adjusted for accession differences (to see how much it explained the variation among replicates within accessions), as well as unadjusted (to ascertain how well it explained differences among accessions).

In the field trial, the number of infected wounds as a proportion of the total number of wounds made on a tree minus the casualties was modelled as a binomial generalised mixed effects model with treatment (rasp wound or leaf scar) and position (central or lateral shoot) as fixed effects and accession as a random effect. The R package glmer was used to run these analyses. The best linear unbiased predictor (BLUP) disease incidence and the area under the disease progress curve (AUDPC) scores were calculated for each progeny and employed for the comparison of leaf scar vs rasp wound treatments and for genetic analysis.

Genetic map and QTL analysis

The genetic linkage map used for detecting genomic regions controlling resistance to EC was based on the published M9×R5 map of Celton et al. (2009), with a few modifications. In brief, the linkage map was extended to 136 segregating individuals, using a subset of markers evenly spanning the genome, whereas the original map was based on 94 individuals. The genetic map spans 21 and 18 linkage groups (LG), comprising 118 and 125 genetic markers for the 'M9' and R5 parents, respectively. Dot plots for allelic association of genetic markers with phenotype were performed using GenStat 17th edition.

QTL analysis was performed separately on the 'M9' and R5 parental maps using the BLUP values extracted from each genotype based on the field phenotype. The MapQTL® 5.0 software (www.kyazma.nl) was used for QTL detection. The LOD significance thresholds at 95% and 99% genome-wide were calculated using 1000 permutations as being 2.5 and 3.0, respectively. QTLs were detected by interval mapping (IM) using steps of 1 cM for each parental map. The most significant markers for these QTLs were then used as co-factors for multiple QTL mapping (MQM) analysis to test for additional QTLs. The variance explained by the QTL was extracted from MapQTL® 5.0.

Whole genome re-sequencing of 'Robusta 5'

Genomic DNA from R5 was used to prepare a library for Illumina® sequencing using the TrueSeq DNA PCR-free kit (Illumina). The library was multiplexed with other *Malus* samples and sequenced in two lanes of Illumina® HiSeq2000 platform in paired-end mode at the Australian Genome Research Facility (Melbourne, VIC, Australia). Sequence data for R5 and re-sequencing data downloaded from NCBI Sequence Read Archive (SRA# SRP075497) for all accessions from Duan et al. (2017) and other unpublished PFR sequence data were mapped to the 'Golden Delicious' Double Haploid v1.1 genome assembly (https://www.rosaceae.org/species/malus/malus_x_domestica/genome_GDDH13_v1.1) using bowtie2 (v2.2.9) (Langmead and Salzberg 2012). Single nucleotide polymorphisms (SNPs) were called using samtools (v1.3.1) 'mpileup' together with bcftools (v1.3.1) 'call' (Li et al. 2009). The resulting SNPs were filtered using bcftools 'filter' to remove low-quality SNPs (QUAL > 30) and those with unexpected supporting read depth (MIN (DP) > 7 & MAX (DP) < 100). SNPs with alleles unique to R5 were searched in this dataset in the genomic region associated with the EC QTL. High-resolution melting polymerase chain reaction (PCR) primers were designed as described by Chagné et al. (2008) and screened over the M9×R5 segregating population for assignment to the linkage map. A single-tube Taqman™ assay was developed from sequence around LG14_31154572 using the on-line Custom Taqman™ Assay Design Tool (www.thermofisher.com), run on a Roche LightCycler and data analysed within Taqman™ Genotyper application (Thermo Fisher Scientific). The assay was then used to screen the M9×R5 mapping population and breeding germplasm to validate the new marker.

Marker validation

The breeding germplasm used for marker validation consisted of 14 F1 and F2 derivatives of R5 evaluated in 2012 and 2016, respectively, which were selected for fire blight resistance, as well as a number of reference cultivars. Up to 20 trees per genotype on 'M9' rootstock were planted as single tree plots (2012) or 12 trees per genotype as three-tree plots (2016) at 30×30 cm spacing in a randomised block design for each experiment at the PFR Motueka research orchard. All trees in the 2012 experiment were inoculated using the leaf scar method only, while in the 2016 experiment, approximately half of the available trees for each genotype were randomly designated for the rasp wound inoculation method and the remainder allocated for leaf scar inoculation. Two inoculations were performed in each experiment at leaf fall in May/June as described for the M9×R5 population experiment. Visual assessments for EC symptoms for the 2012 experiment were made ten times at 10–34-day intervals beginning at 2 August 2013 (8 WAI) until 12 February 2014 (35 WAI), and for the 2016 experiment on 16 August (10 WAI), 3 September (16 WAI), 19 October (20 WAI) and 13 November 2017 (24 WAI) as described above. For the data analysis, disease incidence progression in the 2012 experiment was measured based on cutting of whole shoots with infected lesions since the trees had many shoots, while in 2016, the same approach was taken as described for the M9×R5 family above.

Results

Glasshouse resistance phenotyping

Significant differences in disease incidence, expressed as the proportion of inoculated leaf scars with lesions, were observed among the accessions at both assessment dates of both trials (Supplementary Fig. 2). As expected, the susceptible reference cultivar 'Royal Gala' exhibited a higher disease incidence (0.44 in 2012, 0.27 in 2014) than the majority of the progeny, and the resistant R5 on 'M9' rootstock remained lesion free in both trials (Table 1). 'M9' was scored as susceptible as 'Royal Gala' when evaluated on its own roots (0.32 in 2012, 0.22 in 2014), but was more tolerant when grafted onto 'M9' (0.10 in 2012). 'Monty's Surprise' was scored similarly to grafted 'M9' in that same year. Approximately half the 44 progeny showed a similar disease incidence to R5, with 38.6% remaining lesion free in 2012 (Fig. 1), while in 2014, 29% of the 14 progeny that had not been tested in 2012 remained lesion free. Progenies AJ47 (2012) and AJ119 (2014) were rated most susceptible, with a disease incidence of 0.55 and 0.77, respectively.

In 2014, higher disease incidence was observed at both assessment dates for accessions that had more bud scars than those with more leaf scars ($p < 0.001$), and similarly for trees of the same accession ($p = 0.028$) at 22 weeks after inoculation. However, the ranking of the accessions did not change when incidence estimates were adjusted for the proportions of scars that were leaf scars.

In both glasshouse trials, the ranking of the accessions for disease incidence was nearly identical at the two assessment dates (Spearman $r = 0.96$, $p < 0.001$ in 2012 and $r = 0.99$, $p < 0.001$ in 2014). Association was also observed between the disease incidence rankings in 2012 and 2014 (Spearman $r = 0.53$, $p = 0.014$) when comparing the 13 accessions that were tested in both trials at 25 weeks after inoculation in 2012 and 22 weeks in 2014.

The mean lesion length increased over time and significant differences in lesion length were observed among the accessions at both assessment dates in 2012. However, in the 2014 trial, no significant differences in lesion length were observed among the accessions and the proportions of scars that were leaf or bud scars did not affect lesion length (data not shown).

The ranking of the accessions based on lesion length changed over time in both glasshouse trials, although associations between lesion length at different times were observed (Spearman $r = 0.52$, $p < 0.001$) in 2012 and ($r = 0.71$, $p < 0.001$) in 2014. No correlation was observed between the lesion length ranking in 2012 and 2014 (Spearman $r = 0.23$, $p = 0.12$), when comparing the 13 accessions that were tested in both trials 25 and 22 weeks after inoculation, respectively.

In 2012, a significant association between disease incidence and log lesion length was observed 15 weeks after inoculation (Pearson $r = 0.59$, $p < 0.001$), but there was no linear relationship between disease incidence and log lesion length 25 weeks after inoculation (Pearson $r = 0.28$, $p = 0.12$) (Supplementary Fig. 3) and the same trend was observed in 2014.

Field resistance phenotyping

A striking contrast in disease incidence was found between the two inoculation methods (Table 1, Fig. 2). The BLUP values for the disease incidence following leaf scar inoculations ranged from 0 for AJ86 to 0.789 for AJ119, and for the AUDPC from below 0 for AJ95 to 45.4 for AJ119 (Table 1; Supplementary Table 1), with the susceptible reference cultivar 'Royal Gala' at 0.965 scoring the highest (Table 1). Nearly half (49.2%) of the 122 progeny demonstrated a probability of disease incidence below 0.010 (Fig. 2), i.e. lower than R5, at 0.011 (Table 1). The disease incidence and AUDPC of the rasp wounds was distributed more evenly, ranging from 0.137 and 27.2 for AJ86 to 0.973 and 117.5 for AJ119, respectively (Table 1), with the majority (89.3%) of the progeny showing a disease incidence score over 0.50 (Fig. 2). The parents and the

Table 1 The best linear unbiased prediction values for European canker (*Neonectria ditissima*) disease incidence (proportion infected wounds as a percentage of the number of inoculated wounds) and area under the disease progress curve (AUDPC) for two inoculation methods, leaf scar and rasp wound, in the field, and disease incidence for two leaf scar inoculation events, in 2012 and 2014 observed 25 and 22 weeks after inoculation, respectively, in the glasshouse for the most resistant (AJ86) and susceptible (AJ119 and AJ47) progenies on their own roots, and the parents 'Malling 9' and 'Robusta 5', together with the reference cultivars 'Jonathan', 'Monty's Surprise', 'Golden Delicious' and 'Royal Gala' grafted onto 'M9' rootstock

Accession	Field 2016				Glasshouse	
	Disease incidence		AUDPC		2012	2014
	Leaf scar	Rasp wound	Leaf scar	Rasp wound		
AJ86	0.000124	0.137	− 2.43	27.20	0.00	Not tested
'Robusta 5'	0.022	0.871	9.29	88.27	0.00	0.00
'Jonathan'	0.030	0.880	6.29	103.68	Not tested	Not tested
'Monty's Surprise'	Not tested	Not tested	Not tested	Not tested	0.10	Not tested
Malling 9' ('M9')	0.127	0.835	10.92	91.15	0.32	0.22
AJ47	0.174	0.844	15.86	98.92	0.55	Not tested
'Golden Delicious'	0.472	0.953	16.89	110.68	Not tested	Not tested
'Royal Gala'	0.965	0.941	40.80	112.82	0.44	0.27
AJ119	0.789	0.973	45.44	117.52	Not tested	0.77

three reference cultivars all rated well over 0.80. The large contrast in disease infection rates resulted in a moderate Pearson correlation coefficient of 0.35 in incidence between the two inoculation methods, although the ranking agreed more (Spearman $r = 0.62$; $p < 0.001$). Nevertheless, the correlation between the inoculation methods was high for the most resistant as well as the most susceptible progeny (Fig. 3), with progeny AJ86 rating lowest and AJ119 highest for both inoculation methods (Table 1). The most incongruent progeny were AJ190 and AJ98, which were much more susceptible when inoculated with the leaf scar method than with the rasp wound method. As there was no effect of the position of the inoculation sites, i.e. central leader or lateral shoots, for each inoculation method (data not presented), a combined score was calculated for each individual progeny for the QTL mapping.

Significant associations between the disease incidence in the glasshouse in 2012 and in the field were observed. The Spearman rank correlation coefficient was 0.50 when comparing glasshouse and field leaf scar inoculations, and 0.48 when comparing glasshouse leaf scar inoculations with field rasp wound inoculations. The lowest rating progeny in the field using both inoculation methods, AJ86, was also among the lowest rating progeny in the glasshouse, with no lesions, and the highest rating progeny in the field, AJ119, was also the most susceptible in the 2014 glasshouse test (not tested in 2012). The incongruent progeny AJ98 was ranked as one of the more susceptible progeny using leaf scars in the field, but in the glasshouse in 2012, it was one of the lowest rating progeny, with no lesions.

Initial QTL mapping and subsequent refinement of locus

A single high-effect QTL conditioning field resistance to EC was detected on LG14 of the R5 parental map for the rasp wound inoculation route (Fig. 4a). No significant QTL for the control of EC resistance was detected for the 'M9' parent.

In the initial mapping, the rasp wound QTL explaining 31.3% of the phenotypic variation for the disease incidence was linked to microsatellite marker NZmsMdMYB17 at the bottom of LG14 at a logarithm of odds (LOD) score of 9.26, while the LOD value for the leaf scar phenotyping was 5.79 (22.7% of the variance explained). To further define the locus and obtain a marker suitable for application of marker-assisted selection (MAS), we utilised information on the SNPs underlying the QTL position in R5. Of the total 1,990,623 SNPs called from the combined R5 sequence, a number of those in the genomic region under the QTL controlling EC resistance appeared to be unique compared with those of 251 other apple accessions. Of the 12 high-resolution melting (HRM) primer pairs designed and flanking SNPs in the QTL interval, nine exhibited clear melting profiles that were polymorphic between those of the parents. Two of these were selected for mapping to the region of interest on the R5 genome (Fig. 4a). These were markers LG14_31154572 (forward primer: 5′ GTT TGG AAG ATG CAC GAT CA 3′; reverse: TGG CTT TGG TAA CTG CAG AA 3′) and LG14_32263521 (forward primer: 5′ AAA TGG GAC TTG CGT AAT GC 3′; reverse: GAC GAC AGC TGC GAA ATG TA 3′). Genetic marker LG14_31154572_HRM exhibited the highest LOD score for the disease incidence and AUDPC (10.86 and 11.55, respectively) and the percentage of

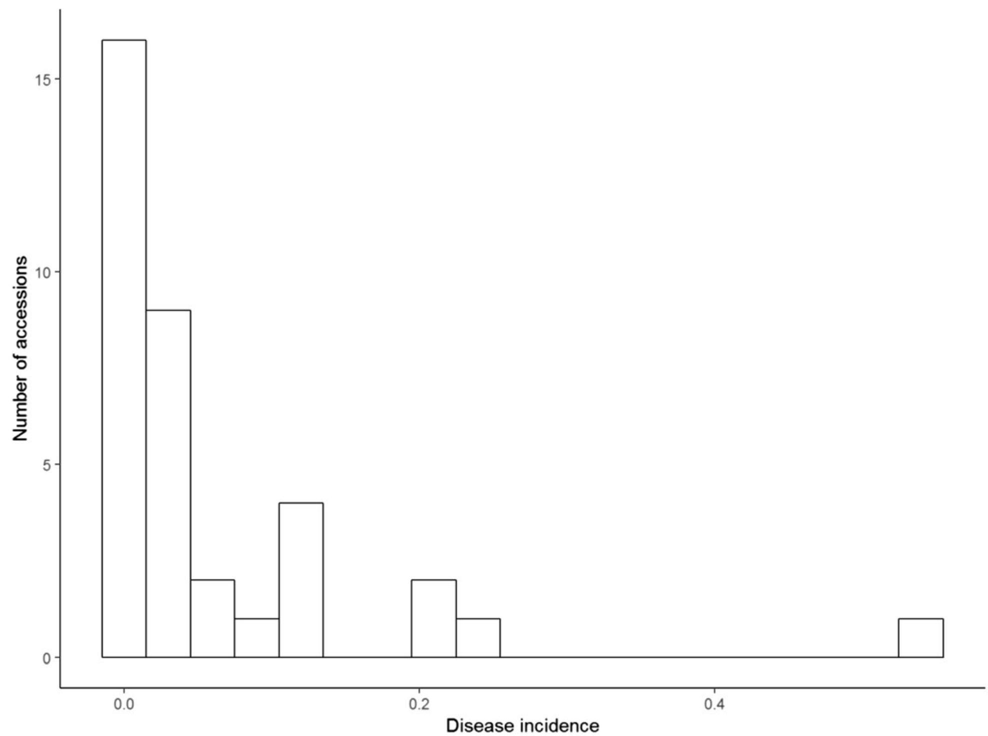

Fig. 1 Disease incidence distribution based on the best linear unbiased predicted values of a 'Malling 9' × 'Robusta 5' progeny phenotyped for European canker (*Neonectria ditissima*) resistance in the glasshouse in 2012, 25 weeks after inoculation onto leaf scars

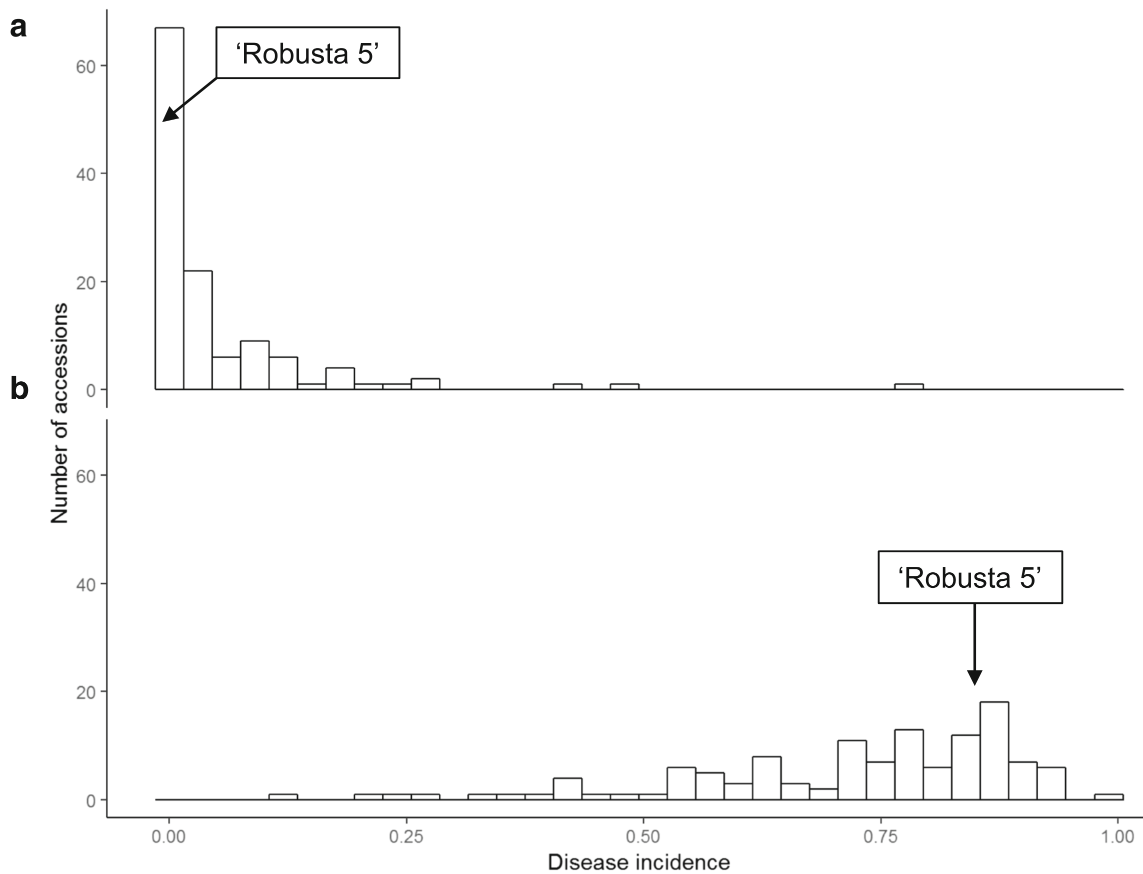

Fig. 2 Disease incidence distribution based on the best linear unbiased predicted values of a 'Malling 9' × 'Robusta 5' progeny phenotyped in the field by inoculation method leaf scar (**a**) or rasp wound (**b**). 'Robusta 5' is positioned as a reference in each histogram based on its scores presented in Table 1

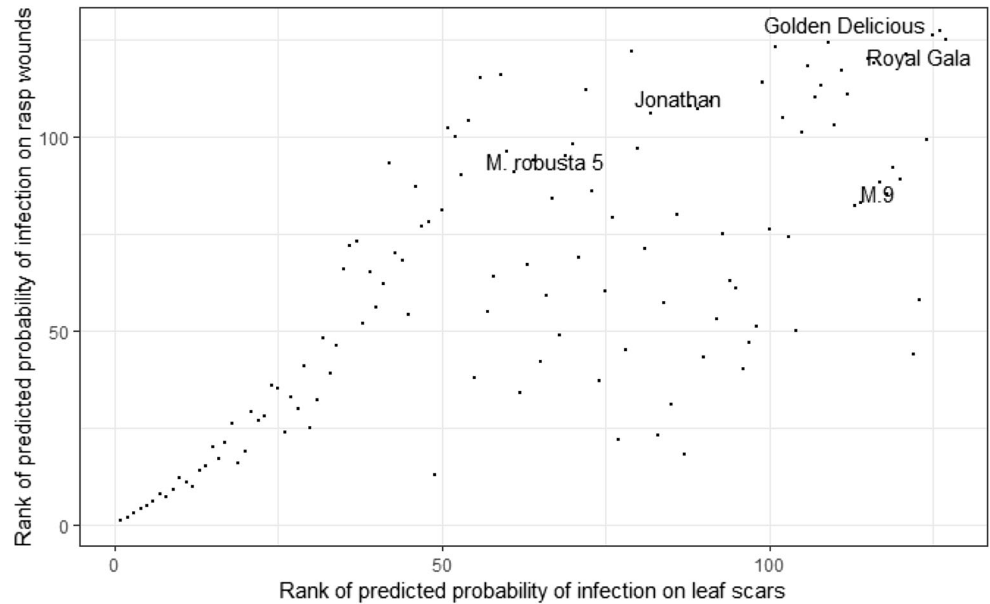

Fig. 3 Scatter plot of the 'Malling 9' × 'Robusta 5' progeny ranked for the best linear unbiased predicted (BLUP) values of the leaf scar and rasp wound treatments for plants phenotyped for European canker (*Neonectria ditissima*) resistance in the field in 2016, including parents and reference cultivars

the phenotypic variance explained by the QTL was 40.7% and 42.2%, respectively. Figure 4b demonstrates the association of the heterozygous genotype for marker LG14_31154572_HRM with a lower disease incidence following rasp wounding as shown by the BLUP values.

In total, 36 and 22 individual plants from the M9×R5 segregating population were phenotyped for EC resistance in the glasshouse in 2012 and 2014, respectively. These numbers were not sufficient to perform a QTL analysis using interval mapping (IM). Hence, the best marker for the QTL on LG14 (LG14_31154572_HRM) controlling resistance in the field was used to calculate, by ANOVA, the effect on the glasshouse-derived phenotypes for EC resistance. Examination of disease incidence over two seasons confirmed that the heterozygous LG14_31154572_HRM allelotype was preferentially, but not exclusively, associated with a reduction in EC symptoms, while it was not associated with lesion length in either year (Supplementary Fig. 4).

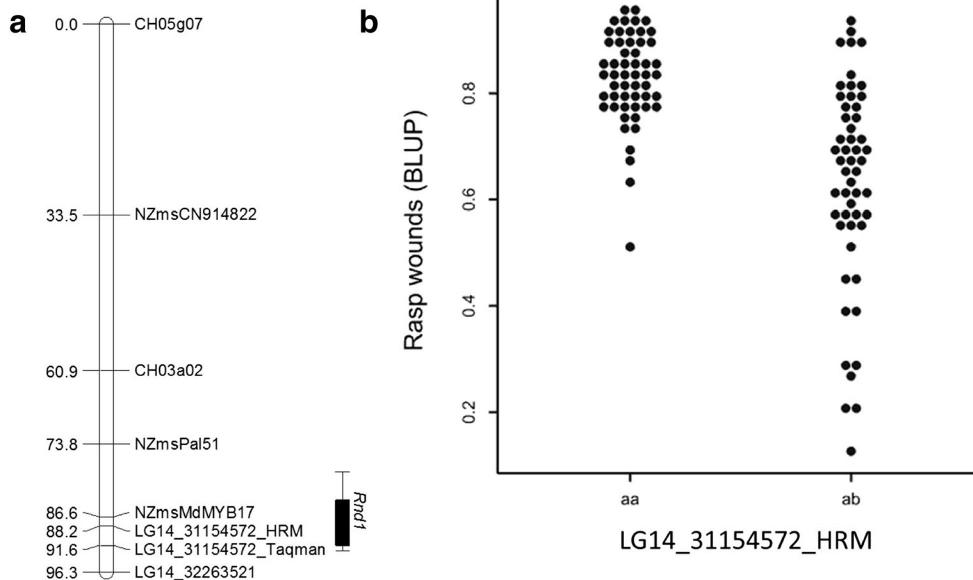

Fig. 4 QTL for resistance to European canker (*Rnd1*) identified on linkage group (LG) 14 of 'Robusta 5', following inoculation by rasp wounds. **a** Linkage and QTL map calculated by interval mapping (IM) using MapQTL® 5.0. Distance in cM is indicated to the left of the map, while the black bar to the right of the map represents a confidence interval at levels of 90% (LOD - 1), and the extended line represents a confidence interval at 95% (LOD - 2). **b** Scatter plot of the high-resolution melting based LG14 QTL: genetic marker LG14_31154572 alleles against rasp wound (BLUP values) inoculation

A highly specific probe-based qPCR Taqman™ marker designed for this position, LG14_3154572_Taqman, mapped close to its HRM version within the QTL interval for *Rnd1*. This assay is available as a custom Taqman™ assay (assay S26033722-S-218, Biosearch Technologies, Petaluma, CA, USA). The marker was evaluated on a small set of breeding germplasm comprising F1 and F2 derivatives of R5 previously selected for fire blight resistance. The leaf scar phenotype data showed that the b allele associated with the *Rnd1* resistance was present in five out of eight EC-tolerant descendants, but only in one of the six susceptible accessions for which phenotype data were available (Table 2). None of the four Cornell-Geneva (CG) apple rootstocks, nor any of the few randomly selected germplasm accessions unrelated to R5, exhibited the marker allele. This included 'Prima', whose two replicate trees were not infected.

Table 2 Disease incidence and marker scores for selected accessions from the breeding germplasm in two independent experiments in 2012 and 2016. The Generation column indicates the generation of the 'Robusta 5' derivatives. The resistance evaluations were performed using the leaf scar inoculation method with the number of inoculated trees presented in brackets. The phenotyped progeny were classed resistant (R) if the incidence leaf scar incidence score was < 1/3 of that of the 'Gala' score in the same year, otherwise susceptible (S). R/S classifications incongruent with the marker score are italicised. The genetic marker was the LG14_31154572 Taqman™ marker for the QTL controlling *Rnd1* resistance to European canker (b allele)

Accession	Generation	2012	2016	Marker
'Robusta 5'		0.05 (17) R	0.00 (6) R	ab
A556R02T117	F2			ab
A556R02T113	F2		0.00 (5) R	ab
A556R02T090	F2			ab
A556R02T085	F2			aa
A556R02T018	F2		0.07 (6) S	aa
A556R01T202	F2		0.13 (2) S	aa
A556R01T198	F2		0.05 (4) *R*	aa
A556R01T139	F2			aa
A556R01T136	F2			ab
A488R05T175	F2		0.02 (5) *R*	aa
A488R05T169	F2		0.11 (2) S	aa
A488R05T150	F2		0.48 (4) S	aa
A414R03T062	F2		0.02 (3) *R*	aa
A414R02T139	F2	0.30 (5) *S*		ab
A414R02T075	F2			aa
A414R02T011	F2			aa
A193R02T034	F1	0.32 (7) S		aa
A191R21T040	F1	0.04 (9) *R*		aa
A190R03T069	F1	0.06 (6) R		ab
A190R02T107	F1	0.00 (8) R		ab
A190R02T037	F1	0.14 (9) R		ab
'CG41'	F1			aa
'CG202'	F1			aa
'CG210'	F1			aa
'CG935'	F1			aa
'Gala' sports		0.44 (17)	0.16 (5)	aa
'Fuji'		0.56 (7)		aa
'PremA129'		0.71 (8)		aa
'Prima'		0.00 (2)		aa
'Aotea'		0.34 (5)		aa
'JM7'				aa
'M116'				aa
Malus calocarpa				aa
M. baccata jackii				aa
M. prunifolia				aa

CG Cornell-Geneva

Discussion

Genetic mapping of the European canker resistance locus Rnd1

This is the first report on the identification of a large-effect QTL for control of resistance to EC in apple. For this purpose, we have used an extensively phenotyped segregating bi-parental population of 122 seedlings derived from a cross made between 'M9' and R5 (Celton et al. 2009), which was employed previously for mapping of other traits (Bus et al. 2008; Peil et al. 2008; Rusholme Pilcher et al. 2008; Gardiner et al. 2012; Foster et al. 2015) and also shown to carry mildew resistance at the *Pl1* locus (Wan and Fazio 2011). The LG14 QTL for control of EC resistance was derived from R5, as were the resistances we mapped earlier for fire blight and woolly apple aphid control on LGs 3 and 17, respectively. This was the sole QTL detected and accounted for a large proportion of the phenotypic variance (42.2%), but since the mapping population was relatively small, this may be an overestimation. The population will need to be extended to clarify this, as well as to identify any lesser impact QTLs that might be carried by 'M9', which exhibits moderate resistance to EC (Moore 1960; Gómez-Cortecero et al. 2016; Scheper et al. 2017).

Our study involved replicated assessment of EC resistance using two methods for inoculation of young trees growing on their own roots in the field, both revealing the same QTL with peak positions for the two phenotypes < 2 cM distant. This finding was supported by the demonstration of the same allelotypic association for the marker mapping at the peak of the QTL for plants grown in the glasshouse and inoculated using leaf scars. Although identification of two major QTLs for control of EC resistance derived from 'Jonathan' was indicated by Van de Weg et al. as reported by Garkava-Gustavsson et al. (2013), this finding has not yet been presented in a full publication. Hence, as the R5 EC resistance is the first QTL to be named, we have assigned the name *resistance to Neonectria ditissima 1* (*Rnd1*) to it.

The *Rnd1* locus maps near the bottom of LG14. To date, only one other resistance locus has been mapped to this linkage group: a small effect, unstable QTL for resistance to powdery mildew in 'Discovery' (Calenge and Durel 2006) at a distance of approximately 8 Mb above *Rnd1*, based on the positions of markers (CH05g11 and NZms*MdMyb17*, respectively) at their respective QTL peaks on the 'Golden Delicious' double haploid genome (Daccord et al. 2017).

Stem wound inoculation with a rasp gave rise to a higher LOD score at the *Rnd1* QTL closest marker for control of resistance than leaf scar inoculation. The difference in phenotypic variance explained by the *Rnd1* QTL (42.2% and 22.7%, respectively) suggests that although the rasp wound inoculation method considerably overestimates plant susceptibility, it can provide effective phenotyping of a population exhibiting transgressive segregation for resistance, as it is capable of differentiating trees at the resistant end of the scale, unlike the leaf scar method (Fig. 2).

A faster method for European canker resistance phenotyping

Phenotyping for EC resistance is commonly recognised to be a laborious process, which now can be accelerated by the use of the rasp wound inoculation technique. Consistent with the findings of Walter et al. (2016), the rasp wound technique involving artificial inoculation applied to central leaders and lateral branches appears to increase inoculation efficiency and/or disease incidence and development rates compared with the techniques based on leaf scars, bud wounds and scalpel wounds (Scheper et al. 2015; Garkava-Gustavsson et al. 2016; Wenneker et al. 2017). In other studies, petroleum jelly was applied as a wound cover to assist the infection process (Van de Weg 1987; Garkava-Gustavsson et al. 2013; Gómez-Cortecero et al. 2016), further adding to an already laborious protocol. Those studies have also suggested that inoculation with a high spore concentration of up to 1000 spores/wound was required. In the present study, the inoculum applied was in the order of 100–300 spores/wound, which was well over the 5 (non-infective) to 50 (infective) spore range (Dubin and English 1974) and the ten spores (Walter et al. 2016) required for infection in the field. Hence, the amount could be reduced further to stretch the use of limited inoculum, provided infection conditions are optimised by, for example, same-day inoculation (Børve et al. 2017). In the glasshouse, misting enabled a “wound wetness period” obviating the need to cover the leaf scars, while in the field, we relied on natural infection conditions, which at the time of inoculation in autumn provided the free moisture required for infection from dew formation on the shoots and/or rain. In light of our finding that the same QTL was identified using either inoculation technique, wounding methods, such as the easy-to-use rasp wound technique, are recommended for EC resistance evaluation experiments in both glasshouse and field screening. At this stage, *in planta* studies will remain standard practice, as the detached shoot assay, while speedy, lacks reproducibility and it appears to have limitations in its application in screening for resistance (Garkava-Gustavsson et al. 2013; Ghasemkhani et al. 2015a).

Although recording only the presence or absence of lesions as a measure of disease incidence over time may somewhat reduce precision, compared with the commonly used time-consuming method of measuring lesion length, it simplifies the efficient handling of large replicated populations. Rasp wounds are quick to make, are readily visible so that their positions on a branch do not require labelling (unlike leaf scars or bud wounds) and are more susceptible to infection and disease development than other wound types (Amponsah et al. 2015; Walter et al. 2016). For example, as few as 2–7% of artificially inoculated leaf and bud scars (without petroleum jelly) developed symptoms in field experiments with ‘Scilate’, ‘Scifresh’ and ‘Royal Gala’ trees (Dryden et al. 2016; Walter et al. 2017), and a large number of inoculation sites were required to obtain adequate data sets. In the study reported here, we used three leaf scars vs one rasp wound per lateral shoot. We found that both parents and the reference cultivars ‘Jonathan’ and ‘Golden Delicious’ were highly infected following rasp wound inoculation. This included R5, which to date, as well as in this study has exhibited very low infection rates with leaf scar inoculations.

Disease incidence was shown to be a robust measure of resistance in this study, as the ranking of the accessions in the two glasshouse trials and in the field were similar, and the ranking of the accessions did not change over time. In contrast, using lesion length, the ranking of the accessions changed over time in both glasshouse trials and the accessions were ranked completely differently in the two trials. Hence, in our experiment, lesion length was not as robust a measure for resistance as disease incidence.

In our study, the *Rnd1* QTL was not associated with lesion length in the glasshouse, consistent with our finding that there was no reliable association between lesion length and disease incidence. This appears to contrast with the findings by Wenneker et al. (2017), who found a high phenotypic correlation between disease incidence and lesion growth rate (LGR) until girdling (rather than lesion length irrespective of girdling), although the statistical significance for disease incidence was higher than for LGR in the resistance separation of germplasm. As we did not record when girdling occurred in our study, calculation of the LGR was not possible. It is known that different phenotyping methods are measures of different aspects of disease development and can result in contrasting germplasm resistance ratings (Ghasemkhani et al. 2015a; Gómez-Cortecero et al. 2016). As our resistance phenotyping focused on disease incidence, the *Rnd1* QTL and underlying genes are expected to be related to establishment of infection rather than disease progression. Measurement of lesion growth rate as well as increasing the family size or mapping in new populations with ‘M9’ or a non-*Rnd1*-resistant derivative of R5 as the major resistance source might enable the identification of additional QTLs in both parents.

Marker-assisted selection

We developed a very specific high-throughput Taqman™ version of the HRM marker LG14_3154572 by targeting SNPs that were unique to R5, using the newly developed bioinformatics tool described above for SNP calling. Its specificity was confirmed in a small sample of non-related apple accessions, including 'Prima', whose two trees in the 2012 germplasm evaluation remained free of symptoms. This agrees with previous research, which showed low disease incidence on this cultivar, but when it did get infected, the colonisation rate was quite high (Garkava-Gustavsson et al. 2016).

We then validated the Taqman™ marker in the mapping population and in selected breeding germplasm. The marker proved highly effective in the mapping population, with strong association of the b allele with resistance. Although the sample of breeding germplasm derived from R5 for which independently collected European canker phenotype data were available was small, the MAS validation largely agreed with the mapping population analysis that additional QTLs to *Rnd1* probably are involved in the resistance. The LG14_3154572 marker allele being present in susceptible plants (Fig. 4b) suggests that the *Rnd1* QTL needs to be combined with other QTLs to effectuate an acceptable level of resistance. In contrast, M9×R5 progenies showing transgressive resistance compared with R5 may well carry 'M9' QTL(s).

In the interim, while we await development for markers for such loci, the *Rnd1* marker can be used as an effective method to screen for the presence of this large effect resistance locus in potential parents. Breeders will also be able to employ this marker to enrich breeding populations for resistant seedlings derived from parents that have been shown in advance to carry *Rnd1*. Phenotypic screening of the reduced population (or preliminary selections from it) will be required to verify the resistance status of individuals with respect to EC resistance before advancing to cultivar status. We have already screened ten F1 and F2 scion breeding families derived from R5 with the Taqman™ *Rnd1* marker, together with markers for the fire blight and woolly apple aphid resistances from this R5 accession (data not presented), the same two traits for which we routinely apply MAS in our apple rootstock breeding programme (Bassett et al. 2015; Bus et al. 2017; Gardiner 2017). In the meantime, research has been initiated to further investigate the genetics of the resistance in the M9×R5 population.

In conclusion, the mapping of the *Rnd1* gene is an initial step towards maximising the durability of resistance to EC in new apple cultivars, based on pyramiding different resistances from multiple sources. Hence, genetic mapping of resistance from a number of sources, such as 'Jonathan' (Garkava-Gustavsson et al. 2013) and 'Aroma' (Gómez-Cortecero et al. 2016), is in progress to identify markers for additional major effect loci for use in MAS. This research is complemented by studies on the genetic variation and host-pathogen interactions of *N. ditissima* to inform breeders on the best strategy for achieving durable resistance.

Acknowledgements The research was funded by Prevar™ and the Strategic Science Investment Fund from the Ministry of Business, Innovation and Employment. We thank Gail Timmerman-Vaughan and Jibran Tahir for critical reading of the manuscript.

Data archiving statement The complete data set for the summary of BLUPS data for the glasshouse and field evaluation of the M9×R5 family provided in Table 1 is presented in Supplementary Table 1. The genetic map of the M9×R5 has been submitted to GDR (https://www.rosaceae.org/node/1539159).

References

Alston FH (1970) Response of apple cultivars to canker, *Nectria galligena*. Rep East Malling Res Station For 1969:147–148

Amponsah NT, Walter M, Beresford RM, Scheper RWA (2015) Seasonal wound presence and susceptibility to *Neonectria ditissima* infection in New Zealand apple trees. N Z Plant Protect 68:250–256

Amponsah NT, Scheper RWA, Fisher B, Walter M, Smits JM, Jesson LK (2017) The effect of wood age on infection by *Neonectria ditissima* through artificial wounds on different apple cultivars. N Z Plant Protect 70:97–105

Bagenal NB (1945) Fruit growing. Modern cultural methods. Ward, Lock & Co. Limited, London, p 416

Bassett H, Malone M, Ward S, Foster T, Chagné D, Bus V (2015) Marker assisted selection in an apple rootstock breeding family. Acta Hortic 1100:25–28

Beresford RM, Kim KS (2011) Identification of regional climatic conditions favorable for development of European canker of apple. Phytopathology 101:135–146

Børve J, Kolltveit SA, Talgø V, Stensvand A (2017) Apple rootstocks may become infected by *Neonectria ditissima* during propagation. Acta Agric Scand Sect B Soil Plant Sci 68:16–25. https://doi.org/10.1080/09064710.2017.1351578

Bus VGM, Chagné D, Bassett HCM, Bowatte D, Calenge F, Celton J-M, Durel C-E, Malone MT, Patocchi A, Ranatunga AC, Rikkerink EHA, Tustin DS, Zhou J, Gardiner SE (2008) Genome mapping of three major resistance genes to woolly apple aphid (*Eriosoma lanigerum* Hausm.). Tree Genet Genomes 4:223–236

Bus VGM, Esmenjaud D, Buck E, Laurens F (2009) Application of genetic markers in rosaceous crops. In: Folta KM, Gardiner SE (eds) Genetics and genomics of the Rosaceae, plant genetics and genomics: crops and models 6, Springer Science+Business Media, p 563–599

Bus V, Singla G, Ward S, Brewer L, Morgan C, Bowatte D, Bassett H, Attfield B, Colhoun C, Bastiaanse H, Walter M, Scheper R, Fisher B, Won K, Montanari S, Volz R, Chagné D, Gardiner SE (2017) Progress in pipfruit resistance breeding and research at Plant & Food Research. Acta Hortic 1172:7–14

Calenge F, Durel CE (2006) Both stable and unstable QTLs for resistance to powdery mildew are detected in apple after four years of field assessments. Mol Breed 17:329–339

Campbell RE, Roy S, Curnow T, Walter M (2016) Monitoring methods and spatial patterns of European canker disease in commercial orchards. N Z Plant Protect 69:213–220

Celton J-M, Tustin DS, Chagné D, Gardiner S (2009) Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from *Malus* ESTs and *Pyrus* genomic sequences. Tree Genet Genomes 5:93–107

Chagné D, Gasic K, Crowhurst RN, Han Y, Bassett HC, Bowatte DR, Lawrence TJ, Rikkerink EHA, Gardiner SE, Korban SS (2008) Development of a set of SNP markers present in expressed genes of apple. Genomics 92:353–358

Cooke LR (1999) The influence of fungicide sprays on infection of apple cv. Bramley's seedling by *Nectria galligena*. Eur J Plant Pathol 105: 783–790

Daccord N, Celton C-M, Linsmith G, Becker C, Choisne N, Schijlen E, van de Geest H, Bianco L, Micheletti D, Velasco R, Di Pierro EA, Gouzy J, Rees DJG, Guérif P, Muranty H, Durel C-E, Laurens F, Lespinasse Y, Gaillard S, Aubourg S, Quesneville H, Weigel D, van de Weg E, Troggio M, Bucher E (2017) High-quality *de novo* assembly of the apple genome and methylome dynamics of early fruit development. Nat Genet 49:1099–1108

Dryden GH, Nelson MA, Smith JT, Walter M (2016) Postharvest foliar nitrogen applications increase *Neonectria ditissima* leaf scar infection in apple trees. N Z Plant Protect 69:230–237

Duan N, Bai Y, Sun H, Wang N, Ma Y, Li M, Wang X, Jiao C, Legall N, Mao L, Wan S, Wang K, He T, Feng S, Zhang Z, Mao Z, Shen X, Chen X, Jiang Y, Wu S, Yin C, Ge S, Yang L, Jiang S, Xu H, L J WD, Qu C, Wang Y, Zuo W, Xiang L, Liu C, Zhang D, Gao Y, Xu Y, Xu K, Chao T, Fazio G, Shu H, Zhong G-Y, Cheng L, Fei Z, Chen X (2017) Genome re-sequencing reveals the history of apple and supports a two-stage model for fruit enlargement. Nat Commun 8:249

Dubin HJ, English H (1974) Factors affecting apple leaf scar infection by *Nectria galligena* conidia. Phytopathology 64:1201–1203

Foster TM, Celton J-M, Chagné D, Tustin DS, Gardiner SE (2015) Two quantitative trait loci, *Dw1* and *Dw2*, are primarily responsible for rootstock-induced dwarfing in apple. Hortic Res 2:15001

Gardiner SE (2017) Novel genetic marker technologies revolutionize apple breeding. Acta Hortic 1174:23–30

Gardiner SE, Norelli JL, da Silva N, Fazio G, Peil A, Malnoy M, Horner M, Bowatte D, Carlisle C, Wiedow C, Wan YZ, Bassett CL, Baldo AM, Celton J-M, Richter K, Aldwinckle HS, Bus VGM (2012) Putative resistance gene markers associated with quantitative trait loci for fire blight resistance in Malus 'Robusta 5' accessions. BMC Genet 13:25

Garkava-Gustavsson L, Zborowska A, Sehic J, Rur M, Nybom H, Englund J-E, Lateur M, van de Weg E, Holefors A (2013) Screening of apple cultivars for resistance to European canker, *Nectria galligena*. Acta Hortic 976:529–536

Garkava-Gustavsson L, Ghasemkhani M, Zborowska A, Englund JE, Lateur M, van de Weg E (2016) Approaches for evaluation of resistance to European canker (*Neonectria ditissima*) in apple. Acta Hortic 1127:75–81

Gelvonauskiene D, Sasnauskas A, Gelvonauskis B (2007) The breeding of apple tree resistant to European canker (*Nectria galligena* Bres.). Sodinink Darzinink 26:174–178

Ghasemkhani M, Liljeroth E, Sehic J, Zborowska A, Nybom H (2015a) Cut-off shoots method for estimation of partial resistance in apple cultivars to fruit tree canker caused by *Neonectria ditissima*. Acta Agric Scand Sect B Soil Plant 65:412–421

Ghasemkhani M, Sehic J, Ahmadi-Afzadi M, Nybom H, Garkava-Gustavsson L (2015b) Screening for partial resistance to fruit tree canker in apple cultivars. Acta Hortic 1099:687–690

Gómez-Cortecero A, Saville RJ, Scheper RWA, Bowen JK, Agripino De Medeiros H, Kingsnorth J, Xu X, Harrison RJ (2016) Variation in host and pathogen in the *Neonectria*/*Malus* interaction; toward an understanding of the genetic basis of resistance to European canker. Front Plant Sci 7:1365

Grove GG (1990) Nectria canker. In: Jones AL, Aldwinckle HS (eds) Compendium of apple and pear diseases. APS Press, St Paul, pp 35–36

Kazlouskaya ZA, Marchuk YG (2013) Evaluation of susceptibility to Nectria canker as initial material for apple breeding. Acta Hortic 976:549–554

Krüger J (1983) Anfälligkeiten von Apfelsorten und Kreuzungsnachkommenschaften für den Obstbaumkrebs nach natürlicher und künstlicher Infektion. Erwerbstobstbau 114:114–116

Langmead B, Salzberg S (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup (2009) The sequence alignment/map (SAM) format and SAMtools. Bioinformatics 25:2078–2079

Moore MH (1934) Some field observations on apple canker (*Nectria galligena*). Rep East Malling Res Station for 1933:166–175

Moore MH (1960) Apple rootstocks susceptible to scab, mildew and canker for use in glasshouse and field experiments. Plant Pathol 9: 84–87

Palm G, Harms F, Vollmer I (2011) Mehrjährige Befallsentwicklung des Obstbaukrebses an verschiedenen Apfelsorten an drei Standorten. Mitt OVR 66:360–363

Peil A, Richter K, Garcia-Libreros T, Hanke MV, Flachowsky H, Celton J-M, Horner M, Gardiner S, Bus V (2008) Confirmation of the fire blight QTL of *Malus* x *robusta* 5 on linkage group 3. Acta Hortic 793:297–303

Rusholme Pilcher RL, Celton J-M, Gardiner SE, Tustin DS (2008) Genetic markers linked to the dwarfing trait of apple rootstock 'Malling 9'. J Am Soc Hortic Sci 133:100–106

Scheper RWA, Stevenson OD, Hedderley (2016) Protection of budding wounds in apple nursery trees from European canker. N Z Plant Protect 69:207–212

Scheper RWA, Frijters L, Fisher BM, Hedderley DI (2015) Effect of freezing of *Neonectria ditissima* inoculum on its pathogenicity. N Z Plant Protect 68:257–263

Scheper RWA, Walter M, Fisher BM, Johnston SL, Curnow T, Amponsah NT, Hedderley DI (2017) Resistance of apple and pear rootstocks to *Neonectria ditissima* and their effect on scion susceptibility. N Z Plant Protect 70:324

Umpleby E, Swarbrick T (1936) The incidence of canker in young cider apple trees. Ann Rep Long Ashton Agric Hortic Res Station for 1935:98–103

Van de Weg WE (1987) Note on an inoculation method to infect young apple seedlings with *Nectria galligena* Bres. Euphytica 36:853–854

Van de Weg WE (1989) Screening for resistance to *Nectria galligena* Bres. in cut shoots of apple. Euphytica 43:233–240

Walter M, Stevenson OD, Amponsah NT, Scheper RWA, Rainham D, Hornblow C, Kerer U, Dryden G, Latter I, Butler RC (2015) Control of *Neonectria ditissima* with copper based products in New Zealand. N Z Plant Protect 68:241–249

Walter M, Roy S, Fisher BM, Mackle L, Amponsah NT, Curnow T, Campbell RE, Braun P, Reinecke A, Scheper RWA (2016) How many conidia are required for wound infection of apple plants by *Neonectria ditissima*? N Z Plant Protect 69:238–245

Walter M, Campbell RE, Amponsah NT, Turner L, Rainham D, Kerer U, Butler RC (2017) Can biological products control *Neonectria ditissima* picking wound and leaf scar infections in apples? N Z Plant Protect 70:63–72

Wan Y, Fazio G (2011) Confirmation by QTL mapping of the *Malus robusta* ('Robusta 5') derived powdery mildew resistance gene *Pl1*. Acta Hortic 903:95–99

Weber RWS (2014) Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a northwestern European perspective. Erwerbs-Obstbau 56:95–107

Wenneker M, Goedhart PW, Van der Steeg P, Van de Weg WE, Schouten HJ (2017) Methods for the quantification of resistance of apple genotypes to European fruit tree canker caused by *Neonectria ditissima*. Plant Dis 101:2012–2019

Wormald H (1955) Diseases of fruits and hops. Crosby Lockwood & Son Ltd, London, p 325

Xu X-M, Butt DJ, Ridout MS (1998) The effects of inoculum dose, duration of wet period, temperature and wound age on infection by *Nectria galligena* of pruning wounds on apple. Eur J Plant Pathol 104:511–519

Zagaja SW, Millikan DF, Kaminski W, Myszka T (1971) Field resistance to *Nectria* canker in apple. Plant Dis Rep 55:445–447

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.