Identification of QTLs for cane splitting in red raspberry (*Rubus idaeus*)

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Abstract

Cane splitting, a normal feature of raspberry growth, can lead to plant infestation by cane midge followed by fungal infection, with losses in yield of up to 50% if left untreated. The extent of splitting in the 'Latham' × 'Glen Moy' reference mapping population was assessed over six years and in three environments and quantitative trait loci (QTL) were identified across linkage groups (LG) 2, 3, 5 and 6. Cane splitting QTL on LG 3 and 5 co-locate with QTL for plant vigour. The cane splitting QTL on LG 6 is associated with the QTL for resistance to root rot caused by *Phytophthora rubi*. Broad sense heritability for cane splitting ranged from 25.6% in 2007 to 49.1% in 2008 in this population. Season and environment were also found to influence cane splitting in this population. Several genes involved in general plant growth and in defence responses lie within these QTL. This is a first step towards identifying the genetic basis of cane splitting in raspberry and the development of genetic markers for use in raspberry breeding programmes.

Keywords

Cane midge, plant vigour, QTL, molecular markers

Abbreviations

BAC Bacterial artificial chromosome

PRR *Phytophthora* root rot

Introduction

During the process of shoot elongation raspberry canes increase in girth as the secondary vascular tissue develops and the periderm layer becomes thicker (Jennings, 1988). The outer cortical cell layers cannot always keep pace with the increase in plant girth and raspberry canes can develop extensive natural longitudinal splits (Jennings, 1988). The time at which the splits occur and the degree of cane splitting observed varies among different cultivars (Seemuller, 1987; Antonin et al. 1998; Gabor, 2006) and although it is considered a normal feature of raspberry growth (Jennings, 1988) the presence of natural splits in raspberry canes allows the plants to become infested by cane midge (*Resseliella theobaldi* (Barnes)). Adults lay eggs in the splits and the emerging larvae eat the cane, causing serious damage to the cane and losses in yield of up to 50% in the following year (Hall et al. 2009). Damaged canes can also become infected with 'midge blight', caused by a wide range of weakly pathogenic fungi and / or the 'cane blight' fungus (*Leptosphaeria coniothyrium*). Control of cane midge is currently achieved through the application of chlorpyrifos, a broad-spectrum insecticide, to the base of primocanes in the spring (Hall et al. 2009) but raspberries are increasingly grown under protected or semi-protected regimes where the numbers of cane midge are high and the number of generations of cane midge per season is increasing (Lole, 2009). In order for raspberry production to be sustainable, particularly in the light of increasing pesticide restrictions, alternative control strategies are desirable and a greater understanding of the factors influencing cane splitting is required.

As yet, it is not clear why the canes of some raspberry varieties split more than others but the strength and / or thickness of cell walls, including those of the outer cortical cells may play a

role. The major component of primary and secondary cell walls is cellulose, which is synthesised by a cellulose synthase complex (reviewed by Taylor, 2008). Secondary cell walls also contain lignin, which has both protective and structural roles and is the product of phenylpropanoid metabolism, which also includes the biosynthesis of suberin, flavonoids and volatile benzenoids. Many of the genes involved in these pathways have been identified in plants (reviewed by Schuurink et al. 2006; Koes et al. 2005) and some have been mapped in raspberry (Kassim et al. 2009; McCallum et al. 2010; Woodhead et al. 2010).

Cane splitting is effectively a naturally occurring wound process and as such the splits and subsequent damage caused by cane midge infestation could be predicted to induce genes involved in the wound response including the emission of volatiles and the induction of hundreds of defence-related genes (reviewed by Wasternack 2007; Browse, 2009). Raspberry plants produce an array of volatile compounds (Robertson et al. 1995). Analysis of volatiles produced in the vicinity of wounded and undamaged raspberry canes has shown increased abundance of a range of related monoterpene compounds, including linalool and geraniol, esters, and products of the volatile benzenoid pathway such as methyl salicylate in wounded plants compared to controls (Shepherd et al. 2009) and several genes involved in volatile biosynthesis have been mapped in raspberry (Woodhead et al. 2010). Some volatile compounds released from recently split raspberry canes are known to strongly attract mated *R. theobaldi* females and are being investigated for inclusion in attractant lures for use in a cane midge control (Hall et al. 2010). Considerable progress has also been made in the discovery of cane midge sex pheromones and their deployment in traps to attract both male and female midges (Hall et al. 2009). This paves the way for effective monitoring of cane midge infestation in raspberry plantations and permits

chemical control measures to be applied more precisely to suit the local conditions. However, if the regions of the raspberry genome associated with cane splitting could be identified, it might be possible to identify markers associated with cane splitting, allowing the identification of germplasm less prone to splitting and therefore less prone to infestation by cane midge.

This approach is now possible in raspberry. The first *Rubus* genetic linkage map was described by Graham et al. (2004), from a widely segregating cross (about 60% similar (Graham and McNicol 1995)) between a European cultivar, 'Glen Moy' (*R. idaeus*) and an American cultivar, 'Latham' (*R. strigosus*). The map has since undergone several revisions as more molecular markers have been added (Graham et al. 2006, 2009, 2011; McCallum et al. 2010; Woodhead et al. 2008, 2010) and there is now a move to employ functional, rather than anonymous, genetic markers to improve the map. In addition many fruit quality traits have been evaluated, including anthocyanin pigments (Kassim et al. 2009), fruit colour (McCallum et al. 2010), plant habit and development characters including cane pubescence and susceptibility to cane diseases (Graham et al. 2006), ripening and cane height (Graham et al. 2009) and raspberry root rot (Graham et al. 2011). This will allow links to be made between genotype and phenotype and the development of robust tools for marker-assisted breeding.

The aim of the present work was to assess the 'Latham' × 'Glen Moy' reference mapping population for cane splitting over 6 seasons and in three environments, to determine the genotype by environment interaction for this trait, assess heritability, locate QTL for cane splitting and identify genetic markers for future screening of the *Rubus* BAC library where appropriate.

Materials & Methods

Plant material and DNA isolations

The mapping population, as described previously (Graham et al. 2004, 2006), consists of a full sib family generated from a cross between the European red raspberry cv. Glen Moy and the North American red raspberry cv. Latham. These varieties differ in a number of key traits including resistance to various diseases and phenological traits such as dormancy requirement and fruit development. The population was planted at three field locations with two open field sites, one of which was clean and the other infected with *Phytophthora rubi,* which causes *Phytophthora* root rot (PRR) in raspberry, (Graham et al. 2011), and one under protection (polythene tunnel). All trials were arranged in a randomised block design with three replicates each containing two replicated plants of 330 genotypes at the clean field site, two replicates of 330 genotypes at the root rot infected site (Graham et al. 2011) and three single-plant replicates of 188 genotypes (randomly selected from the original 330 full sib family for mapping purposes) under polythene tunnel protection (McCallum et al. 2010). DNA was isolated from the parents and progeny (Graham et al. 2003).

Phenotypic data collection for cane splitting

Mapping of this population has focused on two subsets, an initial population MP1 of 94 lines, and a further mapping population of an additional 94 lines (MP2). Phenotypic data on cane splitting was collected on MP1 only in 2003 and 2004 at the clean and infected field sites. In 2005, cane splitting was assessed on the full population of 330 offspring at both the clean and

infected sites and in 2006 and 2007, cane splitting was assessed on 330 offspring for the clean field site only. In 2008, cane splitting was assessed on MP1 and MP2 in the polythene tunnel. The extent of cane splitting was scored on floricanes at either the start or end of the growing season using a 1-5 scale, where 1 represents no splitting and 5 represents severe splitting extending up the canes.

QTL data for comparison

Data regarding cane height (Graham et al. 2009) and root vigour traits, measured as the number of root suckers (density) and the spread of root suckers (diameter) (Graham et al. 2011) were also examined for correlations with cane splitting and to determine if cane splitting was associated with genes of interest.

Plant vigour assessments

Plant vigour, as estimated by the growth of root suckers, was assessed as previously described (Graham et al. 2004). The density and the diameter (i.e. the number and spread of root suckers from the mother plants) were each recorded on a 0-5 scale (0 being no production) in 2003-2006, for the same plants as the cane splitting (although diameter was not measured at the infected site in 2003). The height of the tallest cane was also measured (in cm) for these plants.

Statistical and mapping analysis

The cane splitting scores were analysed using the statistical programme Genstat 12 for Windows to compare the clean and infected sites and to determine the broad sense heritability H^2 for each year. This was estimated as

$$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_e^2)$$

where σ_G^2 and σ_e^2 are the variance components for genotypes and plots. The correlation of the cane splitting scores with the other trait measurements was examined.

QTL mapping was carried out using the MapQTL 5 software (Van Ooijen, 2004). A Kruskal-Wallis test was used as a preliminary test to identify regions of the genome linked to cane splitting for each year, and to explore whether alleles from one or both parents were contributing. A small permutation test was carried out using Genstat to establish appropriate thresholds for the Kruskal-Wallis test. Interval mapping was then carried out using MapQTL. Regression and multiple regression of the cane splitting scores on markers close to the QTL locations was carried out to estimate the percentage trait variance explained by these QTLs.

Results

Analysis of variance of cane splitting and relationship to other traits

Table 1 summarises the cane splitting data for each site. Analysis of variance showed that there was significantly less cane splitting on the infected site than on the clean site in 2003, 2004 and 2005, with $p < 0.001$, $p < 0.05$ and $p < 0.001$ respectively. In addition, the cane splitting scores vary across the different seasons (Table 1). There was no significant interaction between the genotype and the infected/clean site for any year, and so the heritability was calculated from the infected and clean sites together. The broad sense heritability for cane splitting ranged from

25.6% in 2007 to 49.1% in 2008. Genotype means for the mapping population were used for QTL analysis.

Table 2 shows the correlations of the cane splitting scores in 2003-2006 with measurements of root sucker density, root sucker diameter and cane height, based on the progeny means. Apart from 2006, there were significant positive correlations between cane splitting and at least two of these measurements for each year. Fig. 1 shows the relationship between cane splitting and cane height for the clean site in 2005, with less cane splitting on the shorter canes and a larger range of cane splitting scores for the longer canes. Principal coordinates were calculated from the cane splitting scores from 2005-2008 on MP1 and MP2, using a city block similarity. The first principal coordinate explained 34% of the variation. The inverse relationship between this principal coordinate and the first principal coordinate score for root rot in this population (Graham et al. 2011) is shown in Fig 2. Individuals exhibiting lower levels of both cane splitting and root rot are located in the lower left portion of the plot.

QTL analysis

Based on a permutation test with 400 permutations, thresholds for the Kruskal-Wallis (KW) statistic with 1 degree of freedom of 13.0 and 13.8 were selected to correspond to a genome-wide significance of p = 0.1 and 0.05 respectively. For five of the six years, the Kruskal-Wallis analysis showed associations above the 0.05 threshold with markers on LG 3, either those segregating in 'Latham' or those segregating in both parents. For two years, there were associations above the 0.05 threshold with markers segregating in 'Latham' on LG 6 and a further year had an association above the 0.1 threshold. For two years there were significant

associations with markers segregating in ‘Latham’ on LG 5, one above the 0.05 threshold and one above the 0.1 threshold (Fig. 3).

QTL interval mapping was used to estimate the QTL locations more precisely. A permutation test with 1000 permutations indicated a likelihood odds (lod) threshold of 4.2 to correspond to a genome-wide significance of 0.05. A lod threshold of 3.8 corresponds to a genome-wide threshold of 0.1: QTLs with lods between 3.8 and 4.2 will be considered as suggestive. The QTL interval mapping analysis generally confirmed the results of the Kruskal-Wallis analysis, with an additional QTL for cane splitting in 2006 located on LG 2. Restricted multiple QTL mapping (rMQM mapping, (Van Ooijen, 2004)) was also carried out, and showed only one further QTL for cane splitting in 2005 on LG 2 (Fig. 3).

Tables 3a-3d show the mean cane splitting scores associated with the genotypes of four markers chosen from the QTL confidence regions. These markers were not significant according to the genome-wide permutation test for all years, but the signs of the marker effects were consistent. Genotypes with ad at marker bes_Ri29G13R on LG 2 exhibited significantly more cane splitting in two of the six years examined than the other genotypes (Table 3a), and a similar pattern in another year. On LG 3, marker Bac9022SSR01, segregating in both parents and encoding a MYB11-like gene, is in the centre of the QTL confidence interval for four of the five significant years. It has previously been shown to be associated with the most significant QTL for plant vigour as measured by root sucker density and root sucker diameter (Graham et al. 2011); genotype ac, associated previously with high scores for the vigour measurements (Graham et al. 2011), had consistently high scores for cane splitting. Significant effects of alleles from ‘Latham’ at marker RUB98d on LG 5 were found (Table 3c), with genotype aa associated with

more cane splitting and more vigorous plants (Graham et al. 2011). On LG 6, genotype ab at marker ERubLR_SQ11_3_A11 (encoding a putative germin / auxin receptor gene) is associated with a higher level of cane splitting than genotype aa (Table 3d), was previously shown to be associated with lower root rot scores than genotype aa and was associated with plant vigour measures only at the PRR infected site (Graham et al. 2011). Multiple regression on these four markers was found to explain the following percentages of the variation: 2008, 11.6%; 2007, 17.6%; 2006, 22.3%; 2005, 35.7%; 2004, 15.1%; 2003, 23.9%.

Correlations for season 2006 do not follow the general trend and this may be an effect of environmental conditions. There was high rainfall and low mean air temperatures in March, followed by very low rainfall during April and June with long sunshine hours in May and July which may have had an impact on general plant growth. Data on cane height for later years were absent as the trials were maintained as commercial plots and canes were tipped to control plant height.

Discussion

The genetic control of cane splitting in red raspberry has not previously been determined. The study is timely because of the potential of cane splits to become infested with fungal pathogens and cane midge, a pest that is difficult to control by chemical means due to increasing pesticide restrictions, and that is likely to become more problematic due to changing production practises. The identification of four QTL associated with cane splitting marks the first step in determining the factors underlying cane splitting in red raspberry and will permit regions of the genome associated with this trait to be examined in more detail. The aim is to identify genetic markers

associated with a reduced tendency of canes to split that can ultimately be deployed in breeding programmes to develop cultivars with reduced input requirements for more sustainable raspberry production.

Analysis of the 'Latham' × 'Glen Moy' mapping population across 6 years and 3 sites, including one infected with PRR, showed that many progeny exhibited greater levels of cane splitting than either parent and there was an effect of plant vigour on the cane splitting phenotype.

Raspberry plants with greater vigour are more prone to splitting and cane splitting QTL generally co-localise with QTL for measures of plant vigour and PRR. Whether this is entirely due to the outer cortical cell layers on the cane not keeping pace with the increase in plant girth (Jennings, 1988) or is more complex is unclear. In Eucalyptus, faster growing trees, as measured by tree diameter, contained less lignin due to down-regulation of lignin gene transcripts than slower growing trees (Kirst et al. 2004) and similar mechanisms may operate in raspberry. Less vigorous plants may accumulate more lignin and be less prone to splitting, having greater structural strength.

Modest numbers of QTL, accounting for 13% to 27% of the phenotypic growth (reviewed by Kirst et al. 2004), and up to 9.5% of the adaptive variation, in woody perennials have been reported (Jermstad et al. 2003) so the small number of QTL of small effect identified in this study is not unexpected. QTL on LG 3 and LG 5 for plant vigour traits and LG 6 for resistance to PRR (Graham et al. 2011) co-locate with the cane splitting QTL (Fig. 3). The QTL associated with resistance to PRR on LG 3 appears to be a case of high plant vigour, low PRR scores and these are associated with high cane splitting, and the same appears to occur at the height QTL on LG 5 in 2005 (Graham et al. 2011; Fig. 3). However, the QTL associated with PRR on LG 6 is

associated with disease resistance because it was only associated with plant vigour measurements on the PRR infected site (Graham et al. 2011).

On LG 3, the most significant marker within the cane splitting QTL on LG 3 is BAC9O22_SSR 01, an R2R3 MYB family protein. MYB transcription factor proteins play regulatory roles in diverse plant developmental processes, including phenylpropanoid metabolism (Stracke et al. 2001), defence responses (Chen et al. 2006) and cell cycle regulation (Cominelli and Tonelli, 2009). This apparently pleiotropic marker underlies a number of phenotypic traits in raspberry (Graham et al. 2009; 2011) and resides within BAC Ri9O22, which has been sequenced. It contains a number of transcription factors implicated in general plant growth and development (Graham et al. 2011) including a gene encoding an AP2 (APETALA 2) / ethylene response factor (ERF) -type transcription factor which is one of the largest transcription factor families in *Arabidopsis* (Riechmann et al. 2000). The function of some members of this family are associated with actively growing and developing plant tissues, specifying meristematic or division competent states (Nole-Wilson et al. 2005) and maintaining root meristem identity (Aida et al. 2004). A developmentally regulated GTP-binding protein with similarity to a Brevis-radix – like gene involved in root tip proliferation and elongation (Mouchel et al. 2004) and a gene containing RING zinc finger motifs, implicated in plant growth as well as defence (Cheung et al. 2007; Perricone, 2009) are also located in this region.

On LG 6, the most significant marker in the cane splitting QTL is ERubLR_SQ11.3_A11 which encodes an auxin-binding / germin-like protein. These proteins have been proposed to have roles in plant development, fruit ripening (El-Sharkawy et al. 2010), defence against pathogens (Godfrey et al. 2007) and herbivores (Lou and Baldwin, 2006) and contribute to cell wall

structure (Minorsky, 2006). A BAC spanning part of this QTL on LG 6 has recently been sequenced (Graham et al. 2011) and contains transcription factors and meristem-related genes including a clavata 1 (CLV1) precursor, (involved in differentiation of the stem cells in the shoot meristem in Arabidopsis (reviewed by Clark, 2001)) and a knope 4 (KNOX) gene which may be involved in the control of meristem size through modulating cytokinin and gibberellic acid levels (reviewed by Wolters and Jurgens, 2009). The auxin-binding gene ERubLR_SQ11.3_A11 is not present on the BAC but will be used to identify additional BAC clones spanning the cane splitting QTL to further our understanding of the genes involved in this trait.

In light of the importance of plant vigour and cane height in cane splitting, the discovery of genes within the raspberry QTL involved in auxin binding and meristematic activity is not unexpected. As growth continues raspberry canes require greater mechanical support and conducting tissues to maintain robustness and longevity. In plants, this is achieved through the deposition of lignin into cell walls of fibres and tracheary elements, and the formation of phellogen (a meristematic tissue important for cork expansion) and secondary vascular tissue, both of which allow the girth of plant axes to increase (Sehr et al. 2010). Secondary vascular tissue is characterized by the initiation of meristematic activity between primary vascular bundles to produce the vascular cambium. The role of plant hormones in controlling the activity of the vascular cambium has been reported (reviewed by Elo et al. 2009; Sehr et al. 2010) and mechanical stimuli have also been shown to impact on secondary plant growth (Ko et al. 2004). In *Arabidopsis* for example, the weight carried by the stem is the primary signal for cambium differentiation to begin and auxin is the signal transduction molecule for this process (Ko et al. 2004). During the transition from primary to secondary growth in *Arabidopsis* stems induced to

produce woody tissue, 700 genes were differentially expressed and more than 40% of the up-regulated genes were associated with signal transduction and transcriptional regulation (Ko et al. 2004). These included MYB genes, AP2/ERF genes as well as auxin carrier and auxin responsive / binding genes which have also been identified within the raspberry cane splitting QTL on LG 3 and LG 6. The development of a raspberry microarray will allow the expression of these, and many other genes to be evaluated in future work.

Although important, plant vigour is not the only factor controlling cane splitting. There are clear environmental and seasonal effects since the QTL do not explain all the variation observed and some QTL, for example those on LG 2 do not occur in all seasons. Limited sequence information is available for this region on LG 2, and includes a cinnamoyl CoA reductase gene, involved in the first committed step of lignin biosynthesis. Further BAC sequencing will provide new information on the genes present here and perhaps greater insight into the influence of environment on the splitting phenotype. Premature and excessive cane splitting is often observed when periods of cold cause stop/start apical growth of the canes which may or may not be killed, but induces branching of the cane. This is usually accompanied by considerable cane splitting in the basal part of the canes and primocane fruiting raspberry cultivars are particularly prone to this type of damage which provides additional sites for cane midge oviposition (J. Allen, pers. comm.).

This study has highlighted regions of the raspberry genome that are associated with cane splitting and that some of these regions are the same as those implicated in resistance / tolerance to PRR. This has important consequences for the raspberry industry since breeding cultivars that have greater inherent resistance / tolerance to PRR tend to be the most vigorous genotypes with

resistance often derived from cv. Latham (Graham et al. 2011). However, these genotypes may also have the greatest tendency to split and therefore potentially greater susceptibility to cane midge and associated fungal pathogens.

One way forward is to validate the correlation between cane splitting and cane height identified here, to assess if shorter caned genotypes exhibit less cane splitting than longer caned genotypes. Markers accounting for 49% of the variation in cane height have already been identified in the 'Latham' × 'Glen Moy' population (Graham et al. 2009) and are under evaluation in raspberry germplasm accessions. However, high fruit yields have been shown to be associated with fruiting cane architecture traits, particularly lateral length, which alone accounted for 82% of the yield variation (Sønsteby et al. 2009). Lateral lengths increase from the shoot tip towards the base, so cane heights in excess of 2m were required to obtain top yields as the uppermost nodes with short and low-yielding laterals could be removed by tipping (Sønsteby et al. 2009). Thus, reducing cane height to reduce cane splitting may have yield implications. Another option is to deploy other sources of resistance to cane splitting in *Rubus* breeding programmes. *R. crataegifolius*, the Korean raspberry, which grows wild in north-eastern China, Japan, Korea and Russia, has the ability to rapidly repair itself upon cane splitting through the production of a wound periderm (McNicol et al. 1983). The resulting reticulate cane phenotype prevents peeling of the mature primary cortex, rendering it resistant to cane midge attack (McNicol et al. 1983). The segregation of this trait in a new raspberry mapping population is being assessed (N. Jennings, M. Woodhead, K. Smith and J. Graham, unpublished). Together with data from a next generation sequencing project examining genes expressed during wound healing in raspberry (M. Woodhead, N. Jennings, J. Graham, P. Hedley, M. Bayer, unpublished) the search continues

for both greater understanding of the processes involved and robust genetic markers for new cultivar development for sustainable, high quality *Rubus* production.

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Table 1 Summary statistics of the cane splitting scores for the parents and offspring for each year and environment in the *R. idaeus* ('Latham' × 'Glen Moy') mapping population. (med = median, sd = standard deviation, min = minimum of the offspring means, max = maximum of the offspring means and H^2 = broad sense heritability)

Season	**Site**	**Parents**		**Progeny**					H^2
		'Latham' Mean (med)	'Glen Moy' Mean (med)	No.	Mean (med)	sd	Min	Max	
2008	Polytunnel	1 (1)	2.6 (3)	188	1.7 (2)	0.84	1.0	4.0	49.1%
2007	Field	1.3 (1)	1.1 (1)	330	1.4 (1)	0.65	1.0	3.5	25.6%
2006	Field	na	1.8 (2)	330	1.2 (1)	1.05	1.0	4.0	31.2%
2005	Field:Clean	na	1.5 (1)	330	2.5 (3)	1.05	1.0	5.0	47.2%
2005	Field:Infected	1 (1)	1.2 (1)	330	2.2 (2)	1.02	1.0	5.0	
2004	Field:Clean	na	2.6 (2.5)	94	1.9 (2)	0.82	1.0	5.0	32.2%
2004	Field:Infected	ns	1 (1)	94	1.8 (2)	0.75	1.0	4.0	
2003	Field:Clean	na	ns	94	2.2 (2)	0.62	1.0	4.0	35.8%
2003	Field:Infected	1.75 (2)	1.4 (2)	94	1.9 (2)	0.63	1.0	3.5	

na – not applicable; 'Latham' was not planted at the original clean field site in order to reduce any possibility of PRR infection
ns – cane splitting not scored

Table 2 Correlation of mean cane splitting scores of the mapping population with mean scores for root sucker density, root sucker diameter and plant height for 2003-2006. ***, **, * show $p < 0.001, 0.01, 0.05$ respectively

Season	Site	No.	Density	Diameter	Height
2006	Field	188	-0.04^{ns}	-0.04^{ns}	0.14^{ns}
2005	Field:Clean	188	0.17^{*}	0.28^{***}	0.43^{***}
2005	Field:Infected	188	0.22^{**}	0.27^{***}	0.47^{***}
2004	Field:Clean	94	0.28^{**}	0.18^{ns}	0.39^{***}
2004	Field:Infected	94	0.30^{**}	0.36^{***}	0.46^{***}
2003	Field:Clean	94	0.40^{***}	0.35^{***}	0.38^{***}
2003	Field:Infected	94	0.52^{***}	ns	0.50^{***}

ns – not scored

Table 3a Mean cane splitting scores for 2003-2008, associated with the genotypes at bes_Ri29G13R on LG 2. Standard errors are in brackets. R^2 (%) gives the percentage of the trait variance explained by regression on this marker. ***, **, * show $p < 0.001$, 0.01, 0.05 respectively

Trait	ac	ad	bc	bd	R^2 (%)
2008	1.7 (0.10)	2.0 (0.12)	1.6 (0.12)	1.7 (0.13)	2.4^{ns}
2007	1.5 (0.07)	1.6 (0.07)	1.4 (0.07)	1.4 (0.07)	1.0^{ns}
2006	1.5 (0.07)	1.8 (0.08)	1.4 (0.08)	1.5 (0.08)	9.7^{***}
2005	2.4 (0.11)	2.8 (0.12)	2.3 (0.12)	2.6 (0.13)	3.7^{*}
2004	2.0 (0.13)	2.0 (0.13)	1.8 (0.13)	1.7 (0.12)	0.8^{ns}
2003	2.2 (0.10)	2.1 (0.10)	2.1 (0.10)	2.0 (0.09)	0

Table 3b Mean cane splitting scores for 2003-2008, associated with the genotypes at Bac9022SSR01 on LG 3. Standard errors are in brackets. R^2 (%) gives the percentage of the trait variance explained by regression on this marker. ***, **, * show $p < 0.001$, 0.01, 0.05 respectively

Trait	aa	ab	ac	bc	R^2 (%)
2008	1.6 (0.13)	1.7 (0.15)	2.0 (0.09)	1.4 (0.11)	8.0^{**}
2007	1.5 (0.07)	1.3 (0.08)	1.7 (0.06)	1.3 (0.06)	12.3^{***}
2006	1.6 (0.09)	1.4 (0.10)	1.6 (0.07)	1.5 (0.08)	1.8^{ns}
2005	2.4 (0.13)	2.2 (0.14)	2.8 (0.10)	2.3 (0.11)	7.1^{**}
2004	2.1 (0.13)	1.7 (0.14)	2.0 (0.10)	1.7 (0.11)	2.9^{ns}
2003	2.2 (0.10)	1.9 (0.10)	2.3 (0.07)	2.0 (0.08)	12.7^{**}

Table 3c Mean cane splitting scores for 2003-2008, associated with the genotypes at RUB98d on LG 5. Standard errors are in brackets. R^2 (%) gives the percentage of the trait variance explained by regression on this marker. ***, **, *, ‡ show p < 0.001, 0.01, 0.05, 0.1 respectively

Trait	aa	ab	R^2 (%)
2008	1.7 (0.08)	1.6 (0.09)	0^{ns}
2007	1.6 (0.05)	1.3 (0.05)	5.8^{***}
2006	1.6 (0.05)	1.5 (0.06)	2.1^{*}
2005	2.7 (0.08)	2.2 (0.08)	10.6^{***}
2004	2.0 (0.08)	1.8 (0.09)	4.4^{*}
2003	2.2 (0.06)	2.0 (0.06)	8.3^{**}

Table 3d Mean cane splitting scores for 2003-2008, associated with the genotypes at ERubLR_SQ11.3_A11 on LG 6. Standard errors are in brackets. R^2 (%) gives the percentage of the trait variance explained by regression on this marker. ***, **, *, ‡ show p < 0.001, 0.01, 0.05, 0.1 respectively

Trait	aa	ab	R^2 (%)
2008	1.5 (0.09)	1.9 (0.08)	8.3^{***}
2007	1.4 (0.05)	1.5 (0.05)	0.8^{ns}
2006	1.4 (0.06)	1.7 (0.05)	8.2^{***}
2005	2.2 (0.08)	2.8 (0.07)	17.6^{***}

2004	1.8 (0.08)	2.0 (0.08)	6.3*
2003	2.1 (0.06)	2.2 (0.06)	2.0‡

Fig. 1 Correlation between raspberry cane height and cane splitting in 2005 in the 'Latham' × 'Glen Moy' raspberry reference mapping population

Fig. 2 Plot of the first principal coordinate scores calculated from the cane splitting (2005-2008) and root rot scores (2005-2006) from the 188 progeny from the 'Latham' × 'Glen Moy' raspberry reference mapping population. High principal coordinate scores correspond to high cane splitting or root rot scores

Fig. 3 Linkage groups 1-7 showing one-lod confidence intervals (to the right of the LG) for QTL locations for cane splitting, and representative QTL for root rot, root sucker density, root sucker diameter and cane height at clean and root-rot infected (Inf) sites (taken from Graham et al. 2011) in the 'Latham' × 'Glen Moy' raspberry mapping population. Markers significantly associated with the cane splitting QTL and used in the regression analysis are underlined