Theor Appl Genet (2014) 127:1667–1677
DOI 10.1007/s00122-014-2329-4

ORIGINAL PAPER

QTL mapping of black rot (*Guignardia bidwellii*) resistance in the grapevine rootstock 'Börner' (*V. riparia* Gm183 × *V. cinerea* Arnold)

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Received: 22 July 2013 / Accepted: 5 May 2014 / Published online: 28 May 2014

Abstract
Key message **In the grapevine cultivar 'Börner' QTLs for black rot resistance were detected consistently in several independent experiments. For one QTL on chromosome 14 closely linked markers were developed and a detailed map provided.**
Abstract Black rot is a serious grapevine disease that causes substantial yield loss under unfavourable conditions. All traditional European grapevine cultivars are susceptible to the causative fungus *Guignardia bidwellii* which is native to North America. The cultivar 'Börner', an interspecific hybrid of *V. riparia* and *V. cinerea*, shows a high resistance to black rot. Therefore, a mapping population derived from the cross of the susceptible breeding line V3125 ('Schiava grossa' × 'Riesling') with 'Börner' was used to carry out QTL analysis. A resistance test was established based on potted plants which were artificially inoculated in a climate chamber with in vitro produced *G. bidwellii* spores. Several rating systems were developed and tested. Finally, a five class scheme was applied for scoring the level of resistance. A major QTL was detected based on a previously constructed genetic map and data from six independent resistance tests in the climate chamber and one rating of natural infections in the field. The QTL is located on linkage group 14 (*Rgb1*) and explained up to 21.8 % of the phenotypic variation (LOD 10.5). A second stable QTL mapped on linkage group 16 (*Rgb2*; LOD 4.2) and explained 8.5 % of the phenotypic variation. These two QTLs together with several minor QTLs observed on the integrated map indicate a polygenic nature of the black rot resistance in 'Börner'. A detailed genetic map is presented for the locus *Rgb1* with tightly linked markers valuable for the development for marker-assisted selection for black rot resistance in grapevine breeding.

Communicated by Christine A. Hackett.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2329-4) contains supplementary material, which is available to authorized users.

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Introduction

The fungus *Guignardia bidwellii* (Ellis) Viala and Ravaz [anamorph: *Phyllosticta ampelicida* (Engelm.) Aa] is a hemibiotrophic endoparasitic ascomycete that causes grape black rot, a serious disease in warm and humid regions. It can lead to complete fruit loss but is almost irrelevant in dry areas (Ferrin and Ramsdell 1978). Black rot is endemic to eastern North America and is found today in South America, Asia and Europe. The fungus was introduced to Europe with plant material where it was observed for the first time in southern France in 1885 (Ramsdell and Milholland 1988; Pezet and Jermini 1989). Black rot quickly spread throughout Europe though it was recognized as a relevant grapevine disease mostly in Southern France and Italy (Jermini and Gessler 1996; Harms et al. 2005). In the south of Germany black rot became a locally restricted problem in the 1930s and was thus considered as a disease of minor importance without serious economical effects (Lüstner 1935). However, since 2002 severe outbreaks of black rot were reported in various German wine-growing regions (Harms et al. 2005). Moreover, observations in other wine-growing regions indicate that black rot has

become an increasing problem, e.g. in France (D. Merdinoglu, pers. communication) and Hungary (P. Kozma, pers. communication).

European grapevine cultivars (*Vitis vinifera* L.) are highly susceptible to *G. bidwellii* (Demaree et al. 1937) which infects all young green parts of the plant (Agrios 2005). The fungus overwinters in fruit mummies and forms perithecia in spring when the sexual ascospores mature. These cause the primary infection of young tissue. Under warm and humid weather conditions, the infection occurs and ~10–14 days later brown lesions are observed on the leaves together with black pycnidia, the asexual fruit bodies (Reddick 1911; Caltrider 1960; Kuo and Hoch 1996; Hoffman et al. 2002). Necrotic lesions are formed predominantly in parts of the plant that are infected during the growth phase (Jabco et al. 1985). Pycnidia can also be found on the shoots or on the berries, where they spread in a circle until the berries are completely dried up. In addition, asexual spores are formed in the pycnidia, which can in turn infect all parts of the plant (Agrios 2005).

Guignardia bidwellii has become an increasing and serious problem that might be supported by the climatic change providing more favourable conditions for the fungus. Black rot has been shown to accumulate in particular in old and abandoned vineyards, becoming a threat for neighbouring vines (Harms et al. 2005). Concomitantly, due to the reduced fungicide treatments of new cultivars carrying resistances against mildew diseases (Töpfer et al. 2011), these unexpectedly led to a phytosanitary problem when *G. bidwellii* appeared. These new cultivars are either susceptible to black rot or moderately resistant (Loskill et al. 2010) by chance since their selection was focused solely on the two mildew pathogens. To prevent such undesired surprises breeders have started to extend their breeding programs to select plants showing an additional resistance against black rot.

In contrast to black rot several resistance loci for mildew diseases (powdery and downy mildew) have already been described. These loci were found in both American (Dalbó et al. 2001; Akkurt et al. 2007; Welter et al. 2007; Marguerit et al. 2009) and Asian (Blasi et al. 2011; Ramming et al. 2011; Riaz et al. 2011; Schwander et al. 2012) wild *Vitis* species, in the American *Muscadinia rotundifolia* (Merdinoglu et al. 2003; Barker et al. 2005; Blanc et al. 2012), and in *V. vinifera* table grape cultivars cv. 'Kishmish vatkana' as well as in related cultivars (Hoffmann et al. 2008). Up to now 'Kishmish vatkana' and its relatives represent the only example of a mildew resistance in the European *V. vinifera* gene pool. For mildew resistances, molecular markers have already been used successfully for the early selection and combination of resistance traits by applying marker-assisted selection (MAS) in grapevine breeding programs (Eibach et al. 2007). In contrast, for black rot resistance only a single QTL analysis has been described (Dalbó et al. 2000). The authors identified three QTLs using a mapping population of 'Horizon' ('Seyval' × 'Schuyler') × Illinois 547-1 (*V. rupestris* × *V. cinerea* B9). A fine mapping in this material has not yet been reported and mainly RAPD markers were used which make an application in MAS rather difficult. Thus, for breeding programs aiming at the integration of black rot resistance in elite accessions it is highly desirable to develop and use markers tightly linked to the locus for early resistance selection.

The mapping population V3125 × 'Börner', a *V. vinifera* (susceptible) and an interspecific hybrid (resistant), segregates for black rot resistance. It was previously used to localize the genetic determinants for phylloxera root resistance and a framework map based on simple sequence repeat (SSR) markers was available (Zhang et al. 2009). Therefore, this population was chosen to search for QTLs for black rot resistance. As a further prerequisite a reliable method had to be developed for evaluating black rot resistance under controlled conditions as black rot infections are not regularly found at Geilweilerhof vineyards. In contrast to the mildew diseases, a reproducible resistance test like the leaf disc assay for *Plasmopara viticola* has not been established and evaluated for black rot. In this study, we report on the development of an evaluation system and the subsequent QTL analysis for grape black rot resistance.

Materials and methods

Guignardia bidwellii

The fungus was cultivated in Petri dishes (10 cm diameter) on 4 % oatmeal (Kügler Mühle, Siebeldingen, Germany) solidified with 1.5 % agar (Bacto-Agar, BD, Heidelberg, Germany). Propagation of the fungus was performed at 2-week intervals. Twice a year a passage on plants was carried out according to Jailloux (1992). For isolating the fungus from infected leaves, lesions were dissected and dipped into 10 % NaClO for 10 s. Surface-sterilized leaf pieces were then placed on oatmeal agar and incubated at 25 °C and continuous light. For illumination 50 % of the light sources were blacklight (LT58w/073, Narva Lichtquellen GmbH & Co. KG, Brand-Erbisdorf, Germany) plus 50 % of an equivalent mixture of plant lights (Osram 58w/77 Fluora and Osram L58w/965 Biolux, Ritos GmbH, Mömbris, Germany). Conidia were routinely harvested after 17 days as maximum sporulation occured between 14 and 21 days post-inoculation. The mycelium in the Petri dishes was floated with 10 ml of tap water, spores were harvested using a Drigalski spatula and the resulting spore suspension was decanted after 10 min. The concentration of conidia was measured using a Neubauer counting chamber and

adjusted to 10^5 spores/ml. The spore suspension can be stored for up to 14 days at 4 °C without loss of the germination ability.

Two different *G. bidwellii* isolates were used for subsequent tests: Mo05—originally isolated from an infected vine in the Moselle Valley (Molitor and Berkelmann-Loehnertz 2011)—and Gf10, an isolate from infected leaves of cv. 'Regent' found at Geilweilerhof in June 2010.

Plant material

The pseudo-F1 population V3125 ('Schiava grossa' × 'Riesling') × 'Börner' (*V. riparia* Gm183 × *V. cinerea* Arnold) comprises 202 individuals and has been described previously (Zhang et al. 2009). The vines were planted in a vineyard at Geilweilerhof and a single plant protection treatment was conducted after bud burst mid of May (Polyram WG, 0.8 kg/ha, BASF SE, Ludwigshafen, Germany and Naturen Netzschwefel WG, 3.6 kg/ha, BASF SE, Ludwigshafen, Germany). Dormant cuttings were taken from the offspring vines grown in the vineyard at Geilweilerhof in January 2010, rooted, and transferred into a climate chamber (25 °C, 80 % relative air humidity, 12 h photoperiod). The potted vines (least 50 cm in height) were first inoculated with *G. bidwellii* spores after 1 month. Plants were cut down after resistance evaluation in order to induce new shoots. Cuttings were replaced in 2011 if necessary so that three plants per genotype were always available and used for resistance tests.

Phenotyping in climate chamber

Three plants per pseudo-F1-genotype as well as the control plants 'Müller-Thurgau', V3125 (both susceptible) and 'Börner' (resistant) were inoculated at the same time. For inoculation a suspension with *G. bidwellii* conidia was sprayed with a Prima 5 spray unit (GLORIA Haus- und Gartengeräte GmbH, Witten, Germany) on the plants. Per plant 10 ml spore suspension was applied until it dripped off. The temperature was then raised to 30 °C and plants were kept in the dark overnight. In total, up to 600 plants were inoculated at the same time. Six trials (1: June 2010; 2: August 2010; 3: September 2010, 4: March 2011; 5: June 2011; 6: August 2011) were performed in 2 years. For the first trial only the isolate Mo05 was used for infection. To perform trials 2–4 a 1:1 mixture of both strains was applied. Trials 5 and 6 were carried out only with the isolate Gf10.

Disease evaluation

Resistance was evaluated after 14 and 21 days. Nine schemes were developed (Table 1) and tested to evaluate the infected vines. Finally, a five class system as listed in Table 1 was applied to all trials. In addition, the number of leaves attacked by black rot was counted. At the date of inoculation, the five youngest leaves were tagged and those that showed symptoms were counted after 14 and 21 days. Due to the utilization of three vines per genotype, mean values for the results of the five class system and sums of the quantity of infected leaves were calculated for each genotype. Results of each trial were analysed separately to check their reliability. Additionally, one dataset with mean values of all results based on the five class rating scheme was created and used for further QTL analysis (overall data set).

Disease evaluation in the field

During the growing season of 2010, the mapping population became naturally infected in the field. Two plants were scored for each genotype (trial 7). The assessments were conducted on June 28th and August 6th. The four class and five class systems (Table 1) were used for rating.

QTL analysis

For the determination of QTL regions an extended version of the genetic map (Fechter et al. 2014) was computed with the phenotypic data using MapQTL® 6.0 (Van Ooijen 2009). Two different methods were performed. Firstly, an interval mapping (IM) in 1 cM intervals was carried out to detect QTLs. The genome-wide and LG-specific LOD (logarithm of the odds) thresholds for each QTL were calculated using a permutation test with 1,000 repetitions at $\alpha \leq 0.05$ (5 %). Closely flanking markers were then selected as cofactors and a multiple QTL mapping (MQM) performed. Secondly, the non-parametric Kruskal–Wallis rank sum test (KW) was applied with $P = 0.01$ to the global segregation of each locus in order to measure the segregation of QTLs for non-normally distributed data. The P value was estimated using Microsoft Office Excel 2007 and checked against the results of MapQTL® 6.0 (Van Ooijen 2009) to assure the results of KW. A QTL was only considered significant if it was detected by both methods used. Support intervals refer to one-LOD intervals. Further calculations were done with an extended local map.

DNA extraction

Leaf material was collected in cooled 96 deep well plates (ABgene, Epsom, UK). The plates were frozen at −70 °C and leaf material was lyophilized and ground with a TissueLyser mill (Qiagen GmbH, Hilden, Germany). DNA was extracted according to Lemke et al. (2011). Concentration of DNA was defined with a NanoPhotometer Pearl

Table 1 Rating schemes developed to score the degree of resistance of grapevine against *G. bidwellii*

Rating scheme	Calculation and coding
Infection in %	Average value of the infection in % of 5 leaves per plant (max. of 15 data points).
Maximal value	Maximal value of the infection in % per plant.
Infected leaves	Number of infected leaves (max. 15).
Time course	Coding:
	1 = First symptoms 14 days after inoculation
	2 = First symptoms 21 days after inoculation
	3 = No symptoms
Existence of pycnidia	Coding:
	0 = No pycnidia
	2 = Pycnidia
Quality of lesions	Coding:
	0 = No infection
	1 = Bordered lesions
	2 = Ambiguous
	3 = Extensive lesions
Four classes	0 = No macroscopic symptoms
	1 = Necrotic or chlorotic lesions
	2 = Lesions sparse, pycnidia sparse and small size
	3 = Lesions abundant, pycnidia abundant
Five classes	9 = No visible symptoms
	7 = One lesion with a diameter of <2 cm: no pycnidia
	5 = Few lesions with severe pycnidia (<2 cm), one or two leaves show symptoms
	3 = Several lesions with pycnidia on two or more leaves
	1 = Many lesions or extensive lesions up to totally affected leaves with pycnidia on almost all leaves
Two classes	0 = No infection
	1 = Infection

Nine systems were designed and evaluated according to their practicability and applicability for QTL analysis

(Implem, München, Germany) and adjusted to a concentration of 1 ng/µl.

Genotyping

Polymorphisms of simple sequence repeats (SSR or microsatellites) were used to further narrow down QTL regions. Primer pairs (Table S1) were developed based on the 12× genome sequence of PN40024 (Jaillon et al. 2007; Adam-Blondon et al. 2011) available at www.genoscope.de.cns.fr/vitis. The program WebSat (Martins et al. 2009) was used for microsatellite detection and generation of suitable primers on the basis of the Primer3 program (Rozen and Skaletsky 2000) included in the WebSat program. The default setting for the primer design was with an optimal annealing temperature of 60 °C.

Each primer pair developed was validated using the Genoscope blast server (http://www.genoscope.cns.fr/cgi-bin/blast_server/projet_ML/blast.pl). Only primers which corresponded to the desired position in the reference genome PN40024 were used for further analyses. The size of the amplimers varied between 80 and 400 bp, and the respective forward primer was labelled with a fluorescent dye (6-FAM, HEX, TAMRA, or ROX) to perform fragment length determination in a multiplex procedure. Synthesis of primers was done by Metabion, Martinsried, Germany. Usability of the primer pairs was checked in preliminary tests with DNA of parents, grandparents and ten descendants. Only segregating markers were applied to the entire population.

The amplification of markers by PCR was performed with the Type-it Microsatellite® kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Fluorescently labelled primers (0.2 mM) and 1 µl template DNA (1 ng/µl) were applied in each reaction. The PCR was started with a 5-min activation step at 95 °C. Amplification was performed by denaturation at 95 °C for 30 s, annealing at 60 °C for 1 min and an elongation step for 90 s at 72 °C. The cycles run through 30 repetitions. The amplification was completed by a final 30-min elongation step at 60 °C. The sizes of the PCR products were determined using an ABI 3130xl GeneticAnalyzer (Applied Biosystems, Foster

City, USA). The data obtained were analysed using the GeneMapper ®4.0 software (Applied Biosystems, Foster City, USA).

Genetic mapping

Polymorphic markers were integrated in the existing map using JoinMap 4.0 (Van Ooijen 2006). The double pseudo-testcross strategy (Grattapaglia and Sederoff 1994) was carried out with a LOD threshold of 6.0 after evaluating the markers for their goodness-of-fit segregation ratio with a Chi-square test and excluding of samples with more than 20 % of missing values. Map units in centimorgans (cM) were estimated from the Kosambi function (Kosambi 1944). Linkage groups were numbered and orientated following the IGGP nomenclature (Adam-Blondon et al. 2004).

Results

Guignardia bidwellii isolates

In contrast to the biotrophic mildew fungi (*Erysiphe necator* and *P. viticola*), the hemibiotrophic ascomycete *G. bidwellii* can be easily propagated independently from its host plant on oatmeal agar, permitting a continuous production of inoculum. Infection tests showed that the spore suspension can be stored at 4 °C at least for up to 14 days without loss of infectivity. However, differences in growth behaviour on oatmeal agar were recorded for the two isolates Mo05 and Gf10. Mo05 revealed a one-tenth lower number of spores forming approximately 10^4 conidia/ml. Using the new isolate Gf10 a higher spore density of about 10^5 conidia/ml was obtained. In a test mycelia of both isolates did not grow into each other indicating a significant genetic difference between them (data not shown), while two pieces of the same isolate, Gf10, grew without any barrier. No apparent difference between the isolates was monitored after inoculation and outbreak of infection concerning plant susceptibility/resistance.

Phenotyping

In the vineyards at Geilweilerhof, black rot is a limited problem due to low pathogen pressure and highly variable weather conditions throughout the seasons. These unreliable conditions do not permit an effective field selection for resistance within a breeding program. Transferring the trials to the controlled environment of a climate chamber was therefore essential for reproducible and successful evaluation of black rot resistance. Using rooted cuttings in the climate chamber, we were able to carry out three subsequent experiments per season. Thus, in 2010 and 2011 six independent experiments were performed with the complete mapping population V3125 × 'Börner'. The entire population of 202 pseudo-F1 plants, its parents and controls were infected at the same time using three plants per genotype. In addition, the whole population was also phenotyped in the vineyard in 2010 due to weather conditions favourable for natural infection for black rot. For disease evaluation nine rating schemes were developed (Table 1) with all of them providing reliable data for QTL calculations. Using any of the schemes always led to the same major QTL regions. However, differences arose regarding the value of likelihood (LOD value) and the size of support intervals (one-LOD) between the different rating schemes. The five class system (rating: very susceptible 1–3–5–7–9 entirely resistant; see Fig. 1) provided highest LOD values in combination with the smallest one-LOD support intervals (Fig. 2). In addition, this system led to the highest consistency of the scorings from the naturally infected plants in the vineyard with the ratings of the potted vines in the climate chamber. For each individual all 18 ratings (6 trials, 3 replicates) were combined to one overall data point by calculating the average value.

QTL analysis

IM calculations of the results of the trials 3–6 led to a major QTL on chromosome 14 named *Rgb1* (summarized in Table 2). Additionally, MQM delivered a segregating QTL in the same genomic region of chromosome 14 for trials 1 and 2. Calculations with the overall data set provided a LOD value of 7.1 explaining 18.5 % of the phenotypic variance. The marker GF14-04 is linked significantly with the QTL according to Kruskal–Wallis (Table S3). The support interval of the main QTL on the framework map covers the region from 64.3 to 78.1 cM.

Calculations with the results of trials 2, 3, 6 and the overall data set provided a stable QTL on chromosome 16 named *Rgb2* (Table 2). The status of the QTL on linkage group 16 is particularly enhanced by the fact that it was also found using the data of the field evaluation (trial 7). Results of trial 1 delivered the same significant QTL on linkage group 16 using Kruskal–Wallis analysis (Table S3). Both data of the experiments in the climate chamber and the field led to this QTL. Five of seven results showed thus the respective QTL. The LOD value of the QTL is 3.1 using the overall data set and its explanation of the phenotypic variance is 7.3 %. The marker VChr16c_158 was significantly linked with the QTL using Kruskal–Wallis analysis. The one-LOD support interval on the framework map covers the region from 23.4 to 43.2 cM.

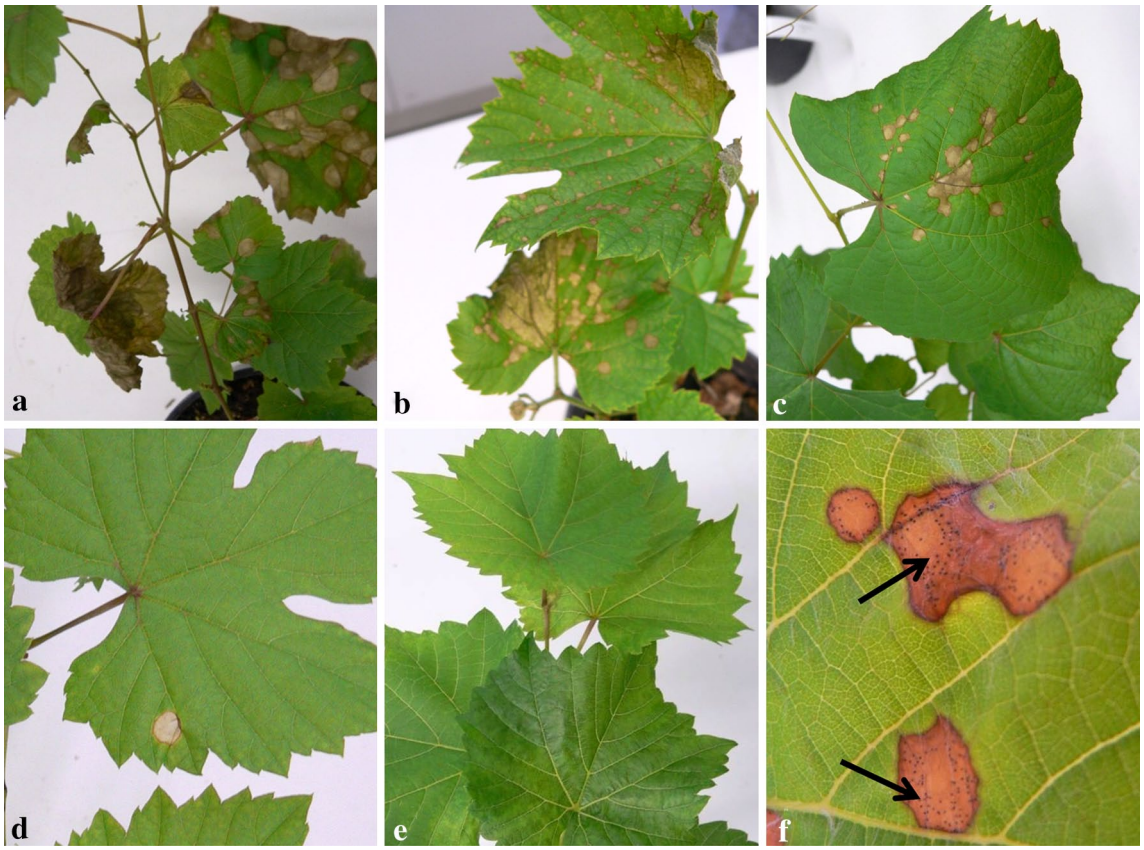

Fig. 1 Necrotic symptoms on grapevine leaves upon black rot infection (21 dpi) representing the five class system (details in Table 1). **a** Very susceptible (rating class 1), **b** susceptible (class 3), **c** moderate (class 5), **d** resistant (class 7) and **e** very resistant (class 9). **f** Pycnidia within necrotic lesions (tiny black spots indicated by *arrows*)

Further QTLs were found on chromosomes 3, 4, 8, 10, 12, 13, and 19 (Table 2). However, these were not reproducible using different data sets.

High-resolution mapping

To reduce the support intervals, new primer pairs for STS (sequence-tagged sites) markers in the region of the QTLs on chromosomes 14 and 16 were developed and tested (Table S1). For chromosome 14, a total of 28 primer pairs were deduced from the reference genome of PN40024 (Jaillon et al. 2007; Adam-Blondon et al. 2011). Out of these, 6 were fully informative in PCR analyses, 13 were partially informative, and 9 delivered no product or were not informative. For chromosome 16, thirty-one primer pairs were designed which resulted in four fully informative markers, five were partially informative, and 22 delivered no product. In summary 60 new primer pairs were designed based on PN40024 of which 49 % could be used for fine mapping in the mapping population V3125 × 'Börner'.

This new genetic map was used together with the overall data set for a further QTL analysis. The results showed that it was possible to narrow down the *Rgb1* locus on chromosome 14. The newly developed marker GF14-42 showed the strongest association with *Rgb1* when using the Kruskal–Wallis analysis. By integrating additional markers, the LOD value of IM increased from 7.1 to 9.8 explaining 21.8 % of the phenotypic variance (Fig. 3). The one-LOD support interval was reduced from 13.8 to 3.4 cM (Table 2) which corresponds to a region of about 2.4 Mb in the reference genome PN40024 (www.genoscope.cns.fr). Moreover, MQM indicates an interval of even <2 cM (74.2–75.8 cM).

The fine mapping on chromosome 16 did not lead to a more defined detection of the position of the *Rgb2* locus. The LOD value is equal to the one obtained with IM in the first map (3.1) and increased only by MQM to 4.2. Major gaps between the marker positions on chromosome 16 resulted in broad support intervals. The marker VChr16c_158 remained linked to the QTL region during fine mapping. The explanation of the phenotypic variance increased slightly from 7.3 to 8.5 % (Fig. S1).

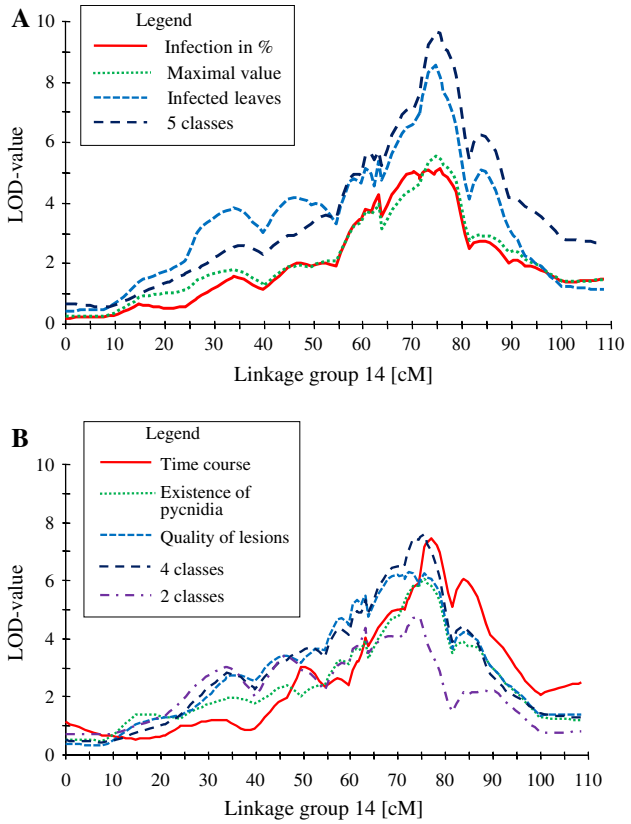

Fig. 2 The QTL on linkage group 14 based on the improved genetic map of V3125 × 'Börner' using nine different rating schemes. **a** Infection in %, maximal value, infected leaves, five classes. **b** Time course, existence of pycnidia, quality of lesions, four classes and two classes. Although the position of the QTL does not change, the five class scale delivers the highest LOD value in combination with a small one-LOD support interval

Discussion

Black rot was considered a minor problem in Europe compared with the mildew diseases. However, since 2002 the number of disease outbreaks has increased drastically in Germany leading to significant yield losses (Molitor and Berkelmann-Loehnertz 2011). In addition to favourable weather conditions in Europe with warmer and more humid summers (Malheiro et al. 2010) two incidents support the increase of black rot infections: a lack of pest management in abandoned vineyards and reduced pest management in vineyards cultivated with mildew-resistant but black rot-susceptible grapevines. These factors support the trend of an increasing black rot pressure on grapevines. Earlier studies on black rot appearance in viticulture in Europe have focussed only on the application of pest management (Loskill et al. 2009; Molitor et al. 2011; Molitor and Berkelmann-Loehnertz 2011). Especially in organic farming, copper in combination with phytosanitary measures seems to be the only method to control infection (Loskill et al. 2009). A long-lasting but consequent approach to address the black rot problem would be to extend breeding programs by defining black rot resistance as a further breeding goal.

In the breeding program at the JKI Institute for grapevine breeding Geilweilerhof black rot selection upon natural infection could not be performed consistently due to unreliable infection conditions in the vineyard in the past. Up to now it was not possible to establish leaf disc assays in analogy to *P. viticola* due to long incubation time and the requirement of actively growing plant tissue (Jabco et al. 1985). Therefore, a resistance screening was established in a climate chamber. We used agar plates for spore production (Rex et al. 2011) as the hemibiotrophic ascomycete *G. bidwellii* requires either young actively growing grapevine tissue or can be cultivated on oatmeal agar. Thus, it became possible to produce spore suspension in large quantities on demand for the artificial inoculation.

Phenotyping in a climate chamber allows the generation of lots of data points within a short period of time. Using this method with artificially infected potted vines, it was possible to run three trials per season, thus minimizing the environmental variance. The use of three plants per genotype proved to be an adequate number of replicates for providing reproducible results. A solid basis for QTL analysis was formed by generating 18 data points per genotype running the experiments six times during 2 years and one field evaluation. Since QTL mapping requires accurate phenotyping data nine different types of scoring the infected plants were tested, e.g. the counting of all leaves showing symptoms and application of three and four class scales (Table 1). In addition to Barrett (1953) and his five class scale, the number of infected leaves and the dimensions of lesions were also recorded. QTL analysis resulted in the same map position for all data sets. The modified five class scale described above was principally designed following the OIV descriptors already available to screen other diseases like powdery or downy mildew (OIV 2009). It might provide a starting point for discussion of an internationally accepted evaluation scale for black rot (Table 1; Fig. 1). Moreover, the five classes permit a comparison of results of different experiments both in the field and in the growth chamber. They include all parts of the symptomatology like the size and quantity of lesions as well as the existence of pycnidia and delivered the highest LOD values in combination with smallest one-LOD support intervals when applied to QTL analysis.

Marker-assisted selection was successfully introduced in grapevine breeding programs a few years ago (e.g. Eibach et al. 2007). It was achieved by applying markers for various sources of powdery and downy mildew resistance as summarized in www.vivc.de (www.vivc.de → 'database search' → 'data on breeding and genetics'). As a

Table 2 Characteristics of the QTLs for resistance to *G. bidwellii* in the consensus map of V3125 × 'Börner'

Trial	LG[a]	LOD_{max} IM	LOD_{max} MQM	LG[b]	Variance explained (%)	Support interval (cM) IM	Support interval (cM) MQM
1	4	4.6	4.0	2.9	12.5	73.5–89.7	[d]
	12	3.6	3.0	2.8	9.9	0.0–9.1	
	14	2.5	3.8	2.9	8.1	64.3–75.0	[d]
	16	–[c]	2.8	2.5	–	–	
	19	3.4	2.7	2.9	9.4	2.5–16.7	[d]
2	**14**	2.5	3.3	3.0	6.8	64.3–75.0	[d]
	15	3.2	3.8	2.6	8.7	0.0–20.8	8.7–18.1
	16	3.6	4.5	2.5	10.1	21.4–43.2	29.1–43.2
3	8	3.4	3.8	2.8	9.2	34.9–46.0	[d]
	14	3.0	4.2	2.9	8.2	55.2–85.6	63.5–75.0
	16	4.1	4.4	2.5	11.2	20.4–43.2	25.1–43.2
4	3	3.4	4.6	2.8	8.9	11.4–36.6	[d]
	14	4.0	3.5	2.9	10.4	45.2–66.1	63.5–74.3
5	13	3.1	3.6	3.0	8.0	7.5–21.2	13.8–18.9
	14	5.7	4.6	3.0	14.8	67.0–79.0	67.0–75.0
6	**14**	7.6	7.0	3.0	19.5	73.3–93.7	71.25–75.0
	16	2.7	3.5	2.4	7.4	24.6–43.2	41.2–43.2
7	10	4.5	5.1	2.9	11.2	34.7–37.3	33.8–37.3
	16	3.2	4.2	2.5	8.2	33.2–43.2	33.2–43.2
Overall data set							
	14	7.1	7.2	2.8	18.5	64.3–78.1	64.3–75.0
	16	3.1	2.8	2.5	7.3	23.4–43.2	22.4–43.2
High-resolution mapping							
	14	9.8	10.5	3.0	21.8	74.6–78.0	74.2–75.8
	16	3.1	4.2	2.8	8.5	24.2–43.2	23.4–43.1

The trial numbers (1: June 2010; 2: August 2010; 3: September 2010, 4: March 2011; 5: June 2011; 6: August 2011; 7: field evaluation 2010) are indicated in the first and the chromosomes for which QTLs were detected in the second column (stable QTLs in bold). For each QTL the LOD score, the threshold and the one-LOD support interval are specified. The table also shows the results of the QTL analysis using the average values of the overall data set before and after the high-resolution mapping

[a] Linkage groups according to the International Grape Genome Program (IGGP) nomenclature

[b] LOD linkage group-specific threshold; estimated value using a permutation test with 1,000 permutations at $\alpha = 0.05$

[c] Missing values are results of data which did not exceed the significance thresholds, but a significance for KW still indicated a QTL (see Table S2)

[d] One-LOD support interval (cM) of MQM does not differ from result of IM

consequence of the focus for mildew diseases, markers for black rot and for various other pests and diseases are demanding. A screening of various parental varieties of the mapping populations available at Geilweilerhof revealed that cv. 'Börner' carries a high black rot resistance. Based on phenotyping of 202 pseudo-F1-individuals and a genetic map constructed using SSR markers, the first detailed QTL for black rot resistance was described. The QTL on linkage group 14 is confirmed by all evaluations in the climate chamber and showed the highest LOD score. This major QTL was named *Rgb1* for *Resistance Guignardia bidwellii.*

The QTL on linkage group 16 was detected in five of seven resistance evaluation trials (Table 2). The status of the QTL was further confirmed by the fact that it was also found using field data (trial 7). This QTL explains 8.5 % of the phenotypic variation and was designated *Rgb2* (LOD 4.2). The low marker coverage in the map of chromosome 16 from Zhang et al. (2009) does not yet provide a clear position of the QTL. Despite several attempts to improve the map of LG 16, up to now a higher resolution could not be established. Most likely the sequence of this chromosome or at least this chromosomal part of 'Börner' differs significantly from the corresponding region of PN40024, making marker design based on the reference sequence more complicated than for other regions.

Further QTLs on linkage groups 3, 4, 8, 10, 12, 13, and 19 were also indicated by the trials, but they were statistically not as significant as the other ones. The number of

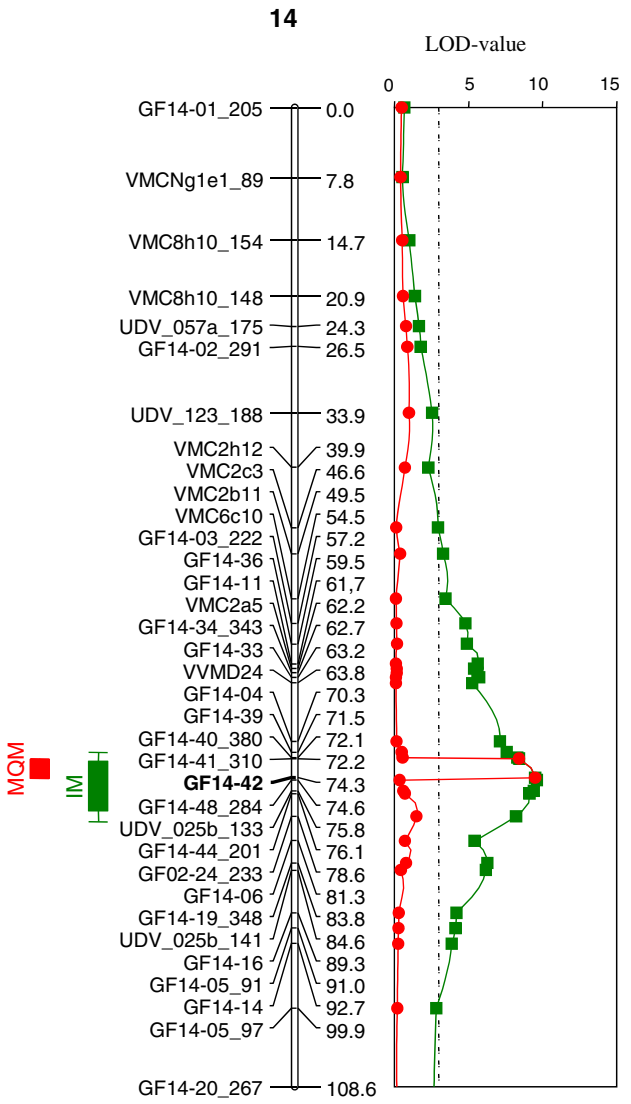

Fig. 3 QTL *Rgb1* for black rot resistance on chromosome 14 analysed with the overall data set after high-resolution mapping. One-LOD support intervals (*bars*) and diagram with the LOD values (*dashed line*) are displayed for interval mapping (*line with squares*) and MQM analysis (*line with circles*). The peaks reached a LOD maximum of 9.8 and 10.5, respectively, and explained 22 % of the variance observed. Marker positions are indicated in cM. The marker used as a cofactor in MQM is highlighted in *bold*

QTLs found clearly shows that 'Börner' carries a polygenic black rot resistance.

At present it is not yet clear whether the two loci, *Rgb1* and *Rgb2*, derive from one or two different genetic sources––namely *V. riparia* and *V. cinerea*, the parents of the black rot resistant 'Börner'. Barrett (1953) tested several wild species for black rot resistance and described *V. cinerea* as a species with the highest type of resistance, where nearly every accession was free of black rot both on foliage or fruit. In the same survey, *V. riparia* was found to be quite variable in resistance to *G. bidwellii.* Heavily infected individuals were observed as well as individuals free of black rot. We assume that the black rot resistance (or at least a part of it) of 'Börner' is probably donated from the North American wild species *V. cinerea* although we cannot exclude *V. riparia* as resistance origin. For *Rgb1* this assumption is indirectly supported by the results of Dalbó et al. (2000) who mapped a QTL for black rot resistance in Illinois 547-1 in the same chromosomal region of LG 14, if the original LG is renamed according to the IGGP nomenclature (B. Reisch, personal communication). Similar to the results of Dalbó et al. (2000, 2001), a QTL for resistance to powdery mildew was also found on 'Börner' chromosome 14, in the vicinity of *Rgb1* (data not shown). It is remarkable that both 'Börner' (*V. riparia* Gm183 × *V. cinerea* Arnold) and Illinois 547-1 (*V. rupestris* × *V. cinerea* B9) are direct descendants of crosses with *V. cinerea*. These two black rot loci might thus be related. Whether the locus found in 'Börner' is identical or allelic to that of Illinois 547-1 remains to be examined in future studies. However, first genotypic marker assays of the region around the QTL on LG 14 revealed that the two black rot resistant cultivars are not identical at the respective genomic site.

Fine mapping of the *Rgb1* locus on chromosome 14 with new markers led to a further size reduction of the IM one-LOD support interval to 3.4 cM. The markers Gf14-41 and Gf14-42 are tightly linked to the resistance locus. To get an idea of the genomic region of *Rgb1* we scanned the corresponding site of the reference genome PN40024 (www.genoscope.cns.fr) for candidate genes. No NBS-LRR gene cluster is located in the region as it is the case for the mildew resistance loci *Ren1* (Hoffmann et al. 2008), *Rpv3* (Bellin et al. 2009) or *Rpv10* (Schwander et al. 2012). However, three single genes coding for proteins with LRR domains are found within this genomic region (GSVIVT01001803001, GSVIVT01001815001, GSVIVT01032422001). In addition, two ankyrin repeat-like proteins (GSVIVT01001817001, GSVIVT01001840001) are located near the markers Gf14-40/41. It was previously demonstrated in *Arabidopsis* that an ankyrin repeat-containing protein is a pivotal regulator of the systemic acquired resistance (SAR) defense response (Cao et al. 1997). Further genes with a putative defense-related function (Jones and Takemoto 2004) were annotated as a chitinase (GSVIVT01032411001), a RIN4-like protein (GSVIVT01003976001), a MAP kinase (GSVIVT01032414001) and an F-box domain containing protein (GSVIVT01001846001). Since the reference variety PN40024 is a black rot-susceptible *V. vinifera* it is tempting to speculate whether functional orthologous genes can be found in the region of *Rgb1* in 'Börner', which are involved in the defense response. Therefore, we have started the sequencing of the *Rgb1* region from 'Börner' which will provide detailed insight into candidate genes and possible resistance mechanisms.

Acknowledgments The authors acknowledge the research funding from the Forschungsring des Deutschen Weinbaus (FDW) and the Federal Ministry of Education and Research (BMBF; Grant No. 0315460B). We also thank Marco Harms (Dienstleistungszentrum ländlicher Raum Rheinpfalz, Neustadt, Germany) for providing the black rot isolate Mo05; Eva Herzog (University Giessen) for her helpful statistical support; Rudolf Eibach, Eva Zyprian, and Florian Schwander for valuable technical advice and fruitful discussions.

Conflict of interest The authors declare that no conflicts of interest exist.

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