RESEARCH ARTICLE

Evaluation of *Rubus* genetic resources on their resistance to cane disease

Vadim Girichev · Marcel von Reth · Magda-Viola Hanke · Monika Höfer · Erik Schulte · Henryk Flachowsky

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Abstract Raspberry cane disease, caused by a complex of fungi, is amongst the most devastating problems for raspberry production. Using resistant or highly tolerant genotypes is a promising strategy. However, until now, cultivars with good field resistance for German fruit growers as well as the primary causal pathogens of cane disease present in the eastern part of Germany, Saxony state are still unknown. The primary objective of this study was to identify the primary disease causing organisms and resistant *Rubus* germplasm for future breeding efforts. From 2012 to 2014, we evaluated the degree of susceptibility to cane diseases on 213 raspberry cultivars at two different locations using a rating scale. We also

identified the main fungi growing on infected canes. Our results suggest that *Fusarium avenaceum* is the main fungus causing cane disease in Saxony. The *Rubus* hybrid cultivars ‘Tayberry’ ($2n = 6x = 42$), ‘Buckingham Tayberry’ ($2n = 6x = 42$) and ‘Dorman Red’ ($2n = 2x = 14$) displayed the highest levels of field resistance at both locations. Moreover, we established an in vitro assay for resistance evaluation which correlates significantly with field data and is particularly suitable for quick assessment of resistance against *F. avenaceum* in breeding material. Future breeding programs, aiming at cultivars with enhanced resistance towards cane diseases can use the presented data for parental selection and may employ our in vitro *F. avenaceum* inoculation method for parental testing and progeny selection.

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Vadim Girichev and Marcel von Reth have contributed equally to this work.

V. Girichev · M. von Reth · M.-V. Hanke ·
M. Höfer · H. Flachowsky (✉)
Julius Kühn-Institut (JKI), Federal Research Centre for
Cultivated Plants, Institute for Breeding Research on Fruit
Crops, Pillnitzer Platz 3a, 01326 Dresden, Germany
e-mail: henryk.flachowsky@julius-kuehn.de

E. Schulte
Federal Office of Plant Varieties, Torgauer Straße 100,
04808 Wurzen, Germany

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Introduction

In 2016, the worldwide production of raspberries amounted to 795,249 t, with about 75% (498,353 t) produced in Europe (Faostat 2016). Currently, main raspberry producing countries are Russia, Poland, and Serbia. In Germany, raspberry production decreased

from 29,700 t in 2002 to 5617 t in 2016, despite the overall increase in the worldwide raspberry production (Faostat 2016). This phenomenon is the result of multiple factors, including high production costs, increased labor costs and an increase in the occurrence of several plant diseases, including black root rot, the spread of fungicide-resistant *Botrytis* strains and cane diseases (Graham et al. 2011; Rupp et al. 2016). Raspberry cane disease is caused by various phytopathogenic fungi, including *Fusarium avenaceum* (*Fusarium* wilt), *Didymella applanata* (spur blight), *Leptosphaeria coniothyrium* (cane blight) and *Botrytis cinerea* (cane *Botrytis*) with *F. avenaceum* being the major cause of cane diseases in Northern Germany (Williamson and Hargreaves 1979; Weber and Entrop 2008). First pink to orange colored spore bearing structures on dark brown lesions which are clear indications of infections of young raspberry primocanes with *F. avenaceum* can be found at the end of May beginning of June at earliest depending on the year and weather conditions (Weber and Entrop 2008). Afterwards disease symptoms are progressing until the end of August, beginning of September.

However, diseased canes in the field are often infected by a complex of several of these fungi, making it difficult to pin down the primary causal pathogen from infection symptoms alone. The infection may be associated with “midge blight” caused by the cane midge *Resseliella theobaldi*. However, this might not necessarily be the case (Williamson and Hargreaves 1979; Weber and Entrop 2008). Hence, we will generalize the disease complex as ‘cane disease’ throughout this manuscript without further specification.

As a result of an infection, fungal pathogens occupy the vascular system and block water and nutrient transport. Infected canes become weakened and die. The consequence is that raspberry plantations quickly become unprofitable, thus emphasizing the need for cultivars with resistance to cane disease. Cultivars combining field resistance to cane disease with superior fruit traits such as large fruit size, sufficient firmness, excellent taste and a beautiful shape are currently not available. Breeding of new and highly resistant cultivars is therefore a major aim in many raspberry breeding programs.

Despite the widespread occurrence of this disease complex and its impact on the raspberry industry, detailed studies concerning the identification of

sources for field resistance in raspberry germplasm collections or in related *Rubus* species are still missing. This includes the identification of the primary causal pathogens in different areas in Germany. Until now, only northern Germany has been assessed (Weber and Entrop 2008). Moreover, artificial inoculation experiments for evaluating the resistance or susceptibility of germplasm have not been established.

Therefore, this study aimed at the identification of resistance sources using a collection of 213 different raspberry cultivars, including *Rubus* hybrid cultivars and other *Rubus* species. Because cane disease occurs in a complex, identification and introgression of pathogen strain-specific resistances into elite cultivars appears extremely difficult. Identification of sources for broad-spectrum or field resistance may be a more promising and durable strategy. *Gene H*, determining pubescence of raspberry canes is a prominent example for broad-spectrum resistance attributed to a single locus in raspberry. It has been shown to be linked with increased resistance towards *Didymella applanata*, *Leptosphaeria coniothyrium* and *Botrytis cinerea* and comes at the cost of enhanced susceptibility towards *Elsinoe veneta* and *Sphaerotheca macularis* (Graham et al. 2006; Jennings 1962, 1982; Jennings and Brydon 1989a, b). Here, we screened the available genetic resources at two different locations in Saxony (East Germany) based on the severity of cane disease symptoms, without attributing symptoms to individual pathogens aiming at the identification of potential sources for broad-spectrum disease resistance. Furthermore, the priority was to identify the primary causal pathogens for Saxony (East Germany), one of the three major fruit production areas in Germany. For this purpose, a field trial was performed from 2012 to 2014 at two different locations. Additionally, we aimed at establishing a reliable inoculation method which allows the identification of resistant germplasm under standardized environmental conditions.

Materials and methods

Plant material and location

In total, 213 different *Rubus* genotypes were evaluated from 2012 to 2014 at two different locations grown according to standard horticultural practices. Most genotypes were red raspberries and belonged to *Rubus*

idaeus L.. In addition the black raspberry (*R. occidentalis* L.) genotype ‘Black Jewel’, the *Rubus* hybrid cultivars ‘Tayberry’, ‘Buckingham Tayberry’, ‘Clen Coe’, and ‘Dorman Red’, as well as the blackberry (*R. fruticosus* L.) cultivar ‘Navaho’ were also included into the study.

Location 1

Borthen, close to Dohna, Saxony (lat 50.968778, long 13.826466). This site is located within a commercial raspberry plantation which is used since many years. ‘Tulameen’ and ‘Clen Ample’ are the two main cultivars which are grown in this plantation. Both cultivars showed always a good level of infection with cane disease. The field trial planted for this study at this site consisted of cultivars commercially used in Germany at this time. Thirty-one *Rubus* cultivars were planted in 2012 in a randomized block design consisting of at least two blocks per cultivar. One block consisted of 20 plants of the same genotype. The cultivars ‘Autumn Bliss’, ‘Lucana’, ‘Meeker’, ‘Niniane’, ‘Octavia’, ‘Malling Minerva’, ‘Royalty’, ‘Rumla’ and ‘Saxa Bliss’ were planted as a single block, because only a small number of plants was commercially available in individual small nurseries. The plantation consisted of five rows and 15 blocks per row. ‘Glen Ample’ (*Rubus idaeus*, red raspberry) is the dominating cultivar for open field production in Germany. Hence, ‘Glen Ample’ was used as a reference cultivar and planted in the first and last blocks of each row and additionally in two random blocks within the plantation. The red raspberry cultivar ‘Tulameen’, known to be affected by cane diseases in Germany, was planted as a susceptible standard in a randomized design in three blocks per row to promote spreading of the naturally occurring fungal causal pathogens of cane disease in this plantation. This plantation was evaluated from 2012 to 2014 (Supplementary Table 1).

Location 2

Wurzen (Saxony) at the small fruit testing station of the Bundessortenamt (lat 51.372439, long 12.757139), 100 km away from location 1. An established genebank collection consisting of 200 different cultivars with single blocks of at least eight plants per genotype was used for evaluation. The collection

exists since more than 10 years at this site and cane disease is well distributed among the cultivars. This genebank collection was evaluated in October of 2013 and 2014 (Supplementary Table 1).

Resistance evaluation in the field

In order to determine the level of cane disease infestation, we established a rating scale (Fig. S1). This scale was based on the percentage of visibly infected area per cane and consists of six classes namely: class 0 (very resistant, no infection), class 1 (resistant, up to 10% infected area), class 2 (medium resistant, 11–25% infected area), class 3 (medium susceptible, 26–50% infected area), class 4 (susceptible, 51–75% infected area) and class 5 (very susceptible, > 76%).

Isolation and morphological characterization of fungal isolates

In October 2013, when the symptoms of cane disease were most pronounced, samples of diseased raspberry canes were randomly taken in the field from locations 1 and 2. In 2014, sampling was expanded to four time points (May, June, August and October) in order to analyze the seasonal distribution of fungi at location 1 (Supplementary Table 1). At each time point ten different places randomly distributed within the location were selected. From each place up to five diseased canes were taken for the isolation of fungal pathogens. Canes were cut into pieces of 3–5 cm and the infected tissue was isolated from lesion margins and placed in Petri dishes on Czapek Dox Agar (Duchefa, Haarlem, Netherlands) supplemented with 200 mg/L streptomycin and 200 mg/L penicillin. The Petri dishes were incubated under continuous light for 2 weeks at 24 °C and 70% relative humidity in a climatic cabinet. Thereafter, single conidiospores of the developing isolates were transferred onto Czapek Dox Agar without antibiotics and incubated for another 2 weeks under the previously described conditions. Single conidiospore isolates were then inspected macroscopically and microscopically.

Molecular identification of fungal species

Fungal genomic DNA was extracted from each single conidiospore isolate using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

The identification of fungal species followed a two-step process. Firstly, PCR was performed using the ITS specific primers namely: ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') according to White et al. (1990). PCR products were purified with the MinElute® PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced. Sequencing was performed by Eurofins MWG Operon (Ebersberg, Germany), using the primers ITS1 and ITS4. The obtained sequences were used to perform a BLAST search in the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) using the blastn function to identify the isolated fungi on the genus level.

Secondly, PCR with species specific primers was performed for *Fusarium* (*F. culmorum*, *F. sambucinum*, *F. oxysporum*, *F. equiseti* and *F. avenaceum*) described by Mishra et al. (2003) as well as *Alternaria alternata* (Konstantinova et al. 2002), *Botrytis cinerea* (Rigotti et al. 2002) and *D. applanata* (Lindqvist-Kreuzer et al. 2003) to identify these fungi on the species level. The primers used are listed in Supplementary Table 2.

Resistance evaluation in vitro

In total, 39 different *Rubus* genotypes (35 *Rubus idaeus* cultivars, 1 *Rubus fruticosus* cultivar and 3 *Rubus* hybrid cultivars) were evaluated in two consecutive years (2013 and 2014). For each genotype, 15 primocane pieces (approximately 10 cm in length and approximately 1.0–1.5 cm in diameter each) were cut and surface sterilized for 1 min with 70% v/v ethanol, then for another 3 min with 5% v/v NaOCl and finally for 30 s with 70% v/v ethanol. To facilitate infection, a 1 cm long injury was made in the center of the canes with a scalpel, followed by inoculating the wound with 5 µL of a *F. avenaceum* conidia suspension (10⁶ conidia/mL) and pricked upright 2 cm deep into full strength MS medium supplemented with vitamins (Duchefa, Haarlem, Netherlands), adding 30 g/L saccharose, 0.3 mg/L benzyl adenine, 0.1 mg/L indole-3-butyric acid, 0.2 mg/L gibberellic acid and 7 g/L agar with a pH value adjusted to 5.8. After 5 days of

incubation at 24 °C, the canes were evaluated with the previously established, rating scale (Fig. S2). Two variables were taken into account to evaluate the resistance. Variable A describes blight symptoms as percentage of necrosis along the site of inoculation. The rating scale for this variable consists of six classes namely: classes 1 (no necrosis, very resistant), 2 (1–10% necrotic area, resistant), 3 (11–30% necrotic area, medium resistant), 4 (31–60% necrotic area, medium susceptible), 5 (61–90% necrotic area, susceptible,) and 6 (≥ 90% necrotic area, very susceptible). Variable B describes the extent of sporulation as percentage of sporulating tissue along the site of inoculation. The rating scale for variable B consists of six classes namely: classes 1 (no sporulation, very resistant), 2 (1–10% sporulation, resistant), 3 (11–30% sporulation, medium resistant), 4 (31–60% sporulation, medium susceptible), 5 (61–90% sporulation, susceptible), and 6 (> 90% sporulation, very susceptible). This experiment was repeated twice for all cultivars.

Flow cytometry

To analyze ploidy levels of germplasm used in this study, flow cytometry was performed according to Meng and Finn (2002).

Statistics

Pearson *r*-correlation was used in order to correlate average ratings for necrosis or sporulation obtained from in vitro inoculation with average field ratings from location 1 using the formula:

$$r = \frac{N \sum xy - \sum(x)(y)}{\sqrt{[N \sum x^2 - \sum(x)^2][N \sum y^2 - \sum(y)^2]}}$$

where *r* = Pearson *r*-correlation coefficient; *N* = number of values in each data set; $\sum xy$ = sum of the products of paired scores; $\sum x$ = sum of *x* scores; $\sum y$ = sum of *y* scores; $\sum x^2$ = sum of squared *x* scores and $\sum y^2$ = sum of squared *y* scores.

The level of significance of the correlation between two parameters was assessed with a Student's *t* test.

Results

Identification and seasonal distribution of cane associated fungi

Cane associated fungi were isolated as described and cultivated as single conidiospore isolates on agar plates. Subsequently all isolates were evaluated macroscopically and microscopically. Isolates representing the same phenotype were subjected to the same group. The partial ITS region of selected isolates per group was sequenced and blasted in the NCBI database. This allowed the assignment of isolates to fungal genera and often also to the most likely species. Species specific primers (if available) were used to verify the sequencing results. The investigated fungal isolates could be assigned to 24 different species and genera, respectively (Fig. S3). Sixteen out of these belonged to the class of Sordariomycetes, six belonged to dothideomycetes, one belonged to leotiomycetes

and one belonged to zygomycetes (Table 1). Isolates belonging to *Leptosphaeria* and *Botrytis* dominated at the beginning of vegetation, whereas *F. avenaceum* and *A. alternata* clearly dominated in summer and autumn (Fig. 1). A comparison of the seasonal occurrence of fungal species on diseased raspberry canes showed that *F. avenaceum*, *Leptosphaeria* sp., *D. applanata* and *Botrytis* sp. were present at either location, whereas other fungal species were only present at one or the other location (Table 1).

Resistance evaluation at location 1 (2012–2014)

In total, 31 cultivars including the interspecific *Rubus* hybrid cultivars ‘Tayberry’, ‘Buckingham Tayberry’, ‘Glen Coe’, ‘Dorman Red’ and the *Rubus occidentalis* cultivar ‘Black Jewel’, were evaluated three times per year during the seasons of 2012–2014. A comparison of the mean value over all 3 years showed that the cultivars ‘Dorman Red’, ‘Tayberry’ and ‘Buckingham

Table 1 Plant associated fungi detected on diseased raspberry canes at locations 1 and 2 in 2013 and 2014

Class	Species	Location 1	Location 2
Sordariomycetes	<i>Bionectria ochroleuca</i> ^a	x	
	<i>Colletotrichum gloeosporioides</i> ^a		x
	<i>Fusarium asiaticum</i> ^a	x	x
	<i>Fusarium avenaceum</i> ^b	x	x
	<i>Fusarium culmorum</i> ^b	x	x
	<i>Fusarium equiseti</i> ^b	x	x
	<i>Fusarium lateritium</i> ^a	x	
	<i>Fusarium oxysporum</i> ^b	x	
	<i>Fusarium poae</i> ^a	x	
	<i>Fusarium sambucinum</i> ^b	x	x
	<i>Fusarium sporotrichioides</i> ^a	x	
	<i>Fusarium tricinctum</i> ^a	x	
	<i>Fusarium venenatum</i> ^a	x	
	<i>Phomopsis</i> sp.	x	x
	<i>Phomopsis quercella</i> ^a	x	
	<i>Truncatella angustata</i> ^a	x	
Dothideomycetes	<i>Alternaria alternata</i> ^b	x	
	<i>Aureobasidium pullulans</i> ^a	x	
	<i>Cladosporium</i> sp.	x	
	<i>Didymella applanata</i> ^b	x	x
	<i>Epicoccum nigrum</i> ^a	x	
Leotiomycetes	<i>Leptosphaeria coniothyrium</i> ^a	x	x
	<i>Botrytis</i> sp.	x	x
Zygomycetes	<i>Mucor circinelloides</i> ^a	x	

^aThese isolates were not unequivocally identified at the species level. They were evaluated macroscopically, microscopically and by sequencing of the partial ITS region. Based on blastn results obtained from the NCBI database the species given here is the most likely species

^bSequencing results were validated by PCR using species specific primers

Fig. 1 Seasonal distribution of individual fungi in the raspberry plantation at location 1, Borthen in 2014. Each pie chart depicts the proportion of individual fungal species identified at defined

time points during the year 2014. The number of samples taken from visually infected canes is given in parentheses (see also Supplementary Table 1)

Tayberry' were very resistant to cane disease at location 1. Other cultivars, such as 'Glen Coe', 'Black Jewel' and 'Gradina' were rated as resistant. 'Sani-belle', 'Malling Minerva', 'Rubaca'/Niniane® and 'Resa'/Lucana® were the most susceptible cultivars at this location (class 3 = medium susceptible). The commercially most important florican cultivars in German raspberry production 'Tulameen' (Score: 2.3) and 'Glen Ample' (Score: 2.6) were rated as medium resistant. The results are summarized in Fig. 2.

Resistance evaluation at location 2 (2013–2014)

Two hundred genotypes (traditional and modern cultivars) were scored for the occurrence of raspberry cane disease in October of each year. At this time point symptoms of cane disease on hardening primocanes are most pronounced. The cultivars 'Black Jewel' (0.5), 'Lowden' (0.7), 'Rubifall' (0.8) and 'Autumn Treasure' (0.8) were rated as very resistant. The most

susceptible cultivars were 'Chiliwak' (3.7), 'Mada-waska' (3.8), 'Canby' (3.9), rated as medium susceptible and 'Delmes' (4.0), rated as susceptible. Similar to the situation at location 1 the cultivars 'Tulameen' and 'Glen Ample' were rated as medium resistant with scores of 2.4 and 2.5, respectively. Results for all raspberry cultivars evaluated at location 2 are summarized in Table 2. We labeled cultivars harbouring *gene H*, which determines cane pubescence. Historical reports attributed resistance towards *B. cinerea*, *D. applanata* and *L. coniothyrium* and susceptibility towards *Elsinoe veneta* and *Sphaerotheca macularis* to the presence of *Gene H* (Graham et al. 2006; Jennings 1962, 1982; Jennings and Brydon 1989a, b). However, *Gene H* positive cultivars were not among resistant cultivars in this survey (Table 2).

Fig. 2 Evaluation on resistance to cane disease (location 1, 2012–2014, see Supplementary Table 1). The rating was done in three consecutive years following natural infection. The Y-axis shows rating values corresponding to the rating scale

In vitro inoculation with *F. avenaceum*

An in vitro assay was established allowing high-throughput evaluation of plants for resistance to *F. avenaceum*. Using this test, 39 different raspberry cultivars as well as two Tayberry cultivars, the blackberry cultivar ‘Navaho’ and the *R. idaeus* × *R. parvifolius* hybrid cultivar ‘Dorman Red’ were evaluated in 2013 and 2014. Rating for the extent of necrosis and sporulation was done according to the ratings defined in Fig. S2. Both Tayberry cultivars ‘Buckingham Tayberry’ and ‘Tayberry’ as well as the blackberry cultivar ‘Navaho’ were mostly devoid of any symptoms with almost no necrotic lesions and a tendency towards sporulation of $\leq 10\%$. No other genotype with similar levels of disease resistance was found. Nevertheless, some cultivars showed a low tendency towards necrosis, for example ‘Glen Ample’, ‘Dorman Red’, ‘Golden Queen’, ‘Valentina’, ‘Fallgold’, ‘Octavia’ and ‘Autumn First’. The extent of sporulation was acceptable for ‘Valentina’, ‘Fallgold’ and ‘Autumn First’, whereas ‘Octavia’ allowed some sporulation (class 3) (Fig. 3).

Of the 39 cultivars tested in vitro, 28 cultivars were evaluated extensively at location 1 from 2012 to 2014. We calculated the correlation between sporulation and necrosis accounted for in the in vitro inoculation test with field ratings from location 1, as well as the

defined in Supplementary Figure 1. Average values for the rating of each cultivar are shown in squares. Maximum and minimum rating values for each cultivar are depicted as triangles and diamonds, respectively

correlation of necrosis with sporulation. Both, sporulation (Fig. 4a) and necrosis (Fig. 4b) are significantly correlated with field data. Moreover, the correlation between necrosis and sporulation is highly significant as well (Fig. 4c).

Determination of ploidy levels

Differences in ploidy levels may impede the success of crosses. Therefore, we assessed ploidy levels of all *Rubus* genotypes available at the Julius Kühn-Institut in Dresden using a flow cytometer. The results are presented in (Table 3). Except for ‘Tayberry’ and ‘Buckingham Tayberry’, all other cultivars, including ‘Dorman Red’ were found to be diploid (Table 3).

Discussion

Genetic resources available for raspberry breeding have not been evaluated on a large scale in terms of resistance or susceptibility to raspberry cane disease occurring in Germany prior to this study. On the other hand, many pathogens account for various cane diseases in different areas throughout the world. To define which pathogens occur on diseased canes in field grown raspberries, either individually or in combination with others, we isolated and identified

Table 2 Evaluation of 200 cultivars at location 2, the Bundessortenamt (Wurzen) from 2013 to 2014

Cultivar	AR	Σ Canes	Σ Plants	Cultivar	AR	Σ Canes	Σ Plants
Black Jewel	0.5	17	6	Bauer 26	2.7	79	14
Lowden	0.7	68	11	Marwes	2.7	61	14
Rubifall	0.8	107	14	Neuheit II	2.7	57	10
Autumn Treasure	0.8	165	28	Caroline	2.7	134	24
TU1	1.1	86	18	Unermüdliche	2.7	84	16
Carmen	1.1	137	27	Baronne de Warve	2.7	121	24
Deutschland 43	1.4	85	16	Zefa3	2.7	49	12
Alpengold	1.4	45	8	Lloyd George	2.7	146	26
Ontario	1.5	95	18	Malling Orion	2.7	97	18
Trent	1.5	84	16	Geneva	2.7	217	36
Heritage	1.5	103	23	Pujallup	2.7	126	27
Schönemann	1.7	107	25	Goliath	2.7	105	16
Festival	1.7	135	20	Boheme	2.7	96	22
Sugana	1.7	134	20	Malling Jewel	2.7	78	12
Glen Doll	1.8	142	22	Malling Exploit	2.7	29	8
Superlativ	1.8	152	22	Rusilva	2.7	222	44
Maurin Makea	1.8	76	14	Reveille	2.8	73	14
Watson Rubi	1.9	71	13	Multiraspa	2.8	85	16
Royalty	1.9	95	18	Biforza	2.8	112	20
The Lafan	1.9	118	19	St. Johann	2.8	134	24
Korbfüller	1.9	108	23	Asker	2.8	179	22
Proma	1.9	77	14	Jatsi	2.8	66	16
Rumla	2.0	171	34	Fertödi Karmin	2.8	102	22
Haida ^a	2.0	107	15	Gevalo	2.8	84	18
Summit	2.0	53	10	Burgund	2.8	33	10
Malling Seedling	2.0	129	23	Reflamba	2.8	53	12
Loganlike	2.0	100	20	Tadmor	2.8	155	22
Fertödi Zamos	2.1	109	20	Wilkran/Framita [®]	2.8	136	22
Glen Fyne	2.1	102	16	Isabell	2.9	129	28
Polana	2.1	99	21	Glen Moy ^a	2.9	38	10
Korfu Wonder	2.1	127	20	Malling Admiral	2.9	145	27
Dulcita	2.1	97	22	Joan Squire	2.9	80	17
Taylor	2.1	101	18	Glen Clova	2.9	89	19
Himbeerquelle	2.1	85	16	Wei-Rula	2.9	236	53
Himberkönigin	2.2	223	33	Streib2a	2.9	124	34
Deep Purple	2.2	51	8	Erika	2.9	146	18
Annamaria	2.2	41	8	Bulgarski Rubin	2.9	130	24
Bois Blank	2.3	60	13	Glen Prosen	2.9	91	20
Comtesse	2.3	217	45	Pokusa	2.9	12	4
Seneca	2.3	133	18	Sumo2	2.9	61	14
Ariadne	2.3	53	12	Kronprinz	2.9	92	17
Milton	2.3	153	22	Clutha	2.9	74	14
Vene	2.3	98	17	Josephine	3.0	166	29
Zenit	2.3	47	11	Rubaca/Niniane [®]	3.0	50	14
Nagymarosi	2.3	141	18	Immertragende von Feldbrunn	3.0	124	20

Table 2 continued

Cultivar	AR	Σ Canes	Σ Plants	Cultivar	AR	Σ Canes	Σ Plants
Holyoke	2.3	48	15	Dinkum	3.0	199	34
Caliber	2.3	70	18	Motoeka	3.0	160	26
Royal Scott	2.3	72	10	Salviraspa	3.0	99	16
Tola	2.3	12	1	Pechts Herbstfreude	3.0	105	22
Matterhorn	2.4	99	18	Nootka ^a	3.0	79	14
Aromquee	2.4	177	33	Pechts Gigant	3.0	65	15
Gigant	2.4	148	21	Rafzaqu/Himbo Top [®]	3.0	38	11
Stuttgart	2.4	133	19	B. Seedling	3.1	131	26
Rutrago/Rucanta [®]	2.4	86	16	Veten	3.1	31	8
Newburgh	2.4	99	16	Redwing	3.1	63	12
Octavia	2.4	31	11	Malahat	3.1	256	50
Gelbe Antwerpen	2.4	95	18	Fallgold	3.1	84	20
Brandywine	2.4	87	14	Brilliance	3.1	117	24
Viking	2.4	87	14	Poranna Rosa	3.1	89	18
Chilcotin ^a	2.4	71	11	Rafzelsa/Himbostar [®]	3.1	36	8
Glen Ample	2.4	254	56	Rode Radboud	3.1	88	14
Selectie Eversdijk	2.4	100	15	Watson/Ruby [®]	3.2	33	8
Tragilo	2.4	92	15	Rumilo	3.2	114	20
Mayfair	2.4	79	15	Gloria	3.2	13	4
Andenken an Paul Camenzind	2.4	67	9	Malling Promise	3.2	123	22
September	2.4	156	25	Carmelina	3.2	181	33
Saxa Record	2.4	97	15	Herbert	3.2	60	13
Julia	2.5	30	8	Malling Leo	3.2	12	3
Malling Landmark	2.5	147	25	Gradina	3.2	53	15
Jochems Roem	2.5	101	13	Geloy	3.2	75	17
Meeker ^a	2.5	271	49	Sirius	3.2	106	20
NZEH	2.5	81	16	Francesca	3.2	41	10
Glen Rosa	2.5	61	14	Titan	3.3	59	18
Zefal	2.5	104	17	Willamette ^a	3.3	58	10
Spica	2.5	65	14	Tambar	3.3	97	19
Polka	2.5	107	17	Hitra	3.3	96	16
Ida	2.5	8	4	Rumiloba	3.3	64	18
Amity	2.5	168	23	Comox	3.3	68	16
Tulameen	2.5	235	59	Preussen	3.3	102	21
Rafzmach/Elida [®]	2.5	53	18	Frosta	3.3	110	22
Norfolk Gigant	2.5	41	10	Dauerpreussen	3.3	134	20
Norprins	2.5	74	12	Metzer Zucker	3.3	67	16
Köstliche Selita	2.5	129	26	Deutschland 1	3.4	94	18
Nordmark	2.5	101	20	Norna	3.4	41	12
Balder	2.6	123	19	Rucami	3.4	49	14
Cardinal	2.6	130	31	Resa/Lucana [®]	3.5	35	11
Lagorai	2.6	95	15	Malling Delight	3.5	43	12
Phyllis King	2.6	67	10	Qualicum	3.5	117	32
Autumn Bliss	2.6	301	51	Varnes	3.5	127	13
Fetödi Venus	2.6	144	25	Gelbe Siebenkugel	3.5	76	14

Table 2 continued

Cultivar	AR	Σ Canes	Σ Plants	Cultivar	AR	Σ Canes	Σ Plants
Zefa2	2.6	105	21	Framita	3.5	26	10
Golden Bliss	2.6	99	17	Riesen Industrie	3.6	81	16
Orange Marie	2.6	61	10	Doppelernte	3.6	52	11
Skeena	2.6	51	10	Fallred	3.6	60	14
St. Peter	2.6	57	12	Bauer 61	3.6	37	6
Joan J	2.6	96	16	Maravilla	3.7	81	18
Tea	2.7	20	6	Chiliwak	3.7	32	6
Saxa Bliss	2.7	82	14	Madawaska	3.8	72	16
Pechts Record	2.7	109	16	Canby ^a	3.9	42	8
Heja	2.7	102	14	Delmes	4.0	41	10

Sampling was performed as shown in Supplementary Table 1

Cultivar, genotype evaluated; AR, average rating; Σ Canes, total number of canes evaluated for this genotype; Σ Plants, total number of plants evaluated

^aCultivars with *Gene H*

24 different fungi from two locations in Saxony (eastern Germany). Twenty-three out of these cane associated fungi were present at location 1, whereas ten out of them were present at location 2, which is 100 km away from location 1. Importantly, pathogens known to be involved in raspberry cane diseases such as *F. avenaceum*, *L. coniothyrium*, *D. applanata* and *Botrytis* sp. were consistently (at all time points) found at both locations. Therefore, fungi found at both locations are more likely to be the primary causal pathogens of cane disease in Saxony. The occurrence of these fungi may depend on many factors, including seasonal fluctuations. Hence, we looked at the composition of fungi occurring on naturally infected raspberry canes in the field at different time points (May, June, August and October) in 2014. Interestingly, we found that incidences of the classical cane disease causing fungi, such as *D. applanata*, *Botrytis* sp. and *Leptosphaeria* sp. decreased during the season, whereas the incidence of *F. avenaceum* increased (Fig. 1). This might be a further indication that this fungal species is mainly responsible for cane disease in eastern Germany. Together with *F. avenaceum*, the incidence of *A. alternata* increased as well and dominated during summer (Fig. 1). Typically, species of the genus *Alternaria* are saprophytes and *A. alternata* has been suggested to be the most common

fungus on the surface of raspberry canes (Ruokola 1982). However, several species of the genus *Alternaria* have been described as plant pathogens. This is particularly true for various pathovars of *A. alternata* that are able to infect more than 100 host plants (Rotem 1994; Thomma 2003). Future studies will reveal, whether *A. alternata* indeed behaves saprophytic on raspberry canes or if some pathovars are capable of infecting raspberry canes and, thus, contribute to cane diseases. On the other hand, *F. avenaceum* has been shown to be the main pathogen causing cane disease in northern Germany, which is thought to occur either independently or in combination with the cane midge *R. theobaldi* (Weber and Entrop 2008). The high proportion of *F. avenaceum* found on diseased raspberry canes, especially in August and October in the present study, suggests that the observation of Weber and Entrop (2008) may hold true for eastern Germany as well. This is in contrast to other European countries, where *F. avenaceum* appears to play a minor role in the development of cane disease and is mainly involved as one pathogen (amongst others) in the midge blight complex (Hall et al. 2009).

Fig. 3 In vitro inoculation of primocane raspberry canes with *Fusarium avenaceum* (2013–2014). Mean values of rating from 2013–2014 are shown. Rating values correspond to the scale defined in Supplementary Figure 2. **A** Occurrence of necrotic

tissue. **B** Sporulation. Average values for the rating of each cultivar are shown as squares. Maximum and minimum rating values for each cultivar are depicted as triangles and diamonds, respectively

Scoring *Rubus* genetic resources for resistance to cane diseases

By using a rating scale, we evaluated 213 raspberry cultivars at two locations in Saxony after natural infection in the field. This collection represents the majority of *Rubus* germplasm available to raspberry breeding in Germany. In both locations, it was apparent that possible sources for resistance were mainly found in *Rubus* hybrid cultivars (e.g. ‘Dorman

Red’, ‘Glen Coe’, ‘Tayberry’ or ‘Buckingham Tayberry’) or closely related *Rubus* species (*Rubus occidentalis* with the cultivars ‘Lowden’ and ‘Black Jewel’) (Fig. 1, Table 2).

It is difficult to give authoritative reasons behind the broad susceptibility towards cane diseases observed in *R. idaeus* cultivars. One major reason for the lack of resistance sources within *R. idaeus* might be a consequence of the genetic erosion during domestication and the genetic similarity of today’s

Fig. 4 Correlation of in vitro parameters with field data from location 1, Borthen and between in vitro parameters. Rating values of 28 cultivars rated at location 1, Borthen (2012–2014) and in in vitro-inoculation experiments (2013–2014) were used. y = function of the linear regression; R^2 = coefficient of determination; r = correlation coefficient (Pearson). H_0 = testing hypothesis = both parameters are statistically insignificantly correlated. H_A = alternative hypothesis = a statistically

significant correlation between both parameters exists. **a** correlation between sporulation (in vitro) and rating (Borthen). $p < 0.01$ ($p = 0.0003$), the correlation is significant. **b** correlation between necrosis (in vitro) and rating (Borthen). $p < 0.01$ ($p = 0.003$), the correlation is significant. **c** correlation between necrosis (in vitro) and rating (Borthen). $p < 0.01$, ($p = 0.000000001$) the correlation is significant

raspberry cultivars (Dale et al. 1993; Jennings 1988). This can explain why sources of resistance are mainly found in closely related *Rubus* species or their hybrid offspring.

Additionally, historical reports showed that raspberry cultivars with pubescent canes (Genotype *Hh*) were more resistant towards cane diseases caused by *B. cinerea*, *D. applanata* and *L. coniothyrium* (White et al. 1990). In this study, cultivars carrying gene *H* (Jennings and McGregor 1988) ranged from 2.0 ('Haida'), to 3.9 ('Canby') for cane disease resistance (Table 2). Hence, gene *H* appears to have little influence on the degree of field resistance towards cane disease in the tested field plots. These results support our speculation that *D. applanata*, *B. cinerea* and *L. coniothyrium* might play a minor role in the manifestation of cane diseases in Saxony.

Cane splitting of variable intensities are frequently found in raspberry cultivars, and wounds might represent entry sites of various fungi or the cane midge *Reselliella theobaldi*, which can promote midge blight (Vétek et al. 2006; Woodhead et al. 2013). Therefore, part of the susceptibility might be caused by a cultivar's propensity for cane splitting (Woodhead et al. 2013).

Since natural infection is strongly influenced by environmental cues, we developed inoculation tests

that allow controlled and directed infections. A major advantage of these methods is the quick assessment with individual pathogens under a standardized environment. Our in vitro testing method developed for this study circumvents the need of naturally occurring cane splitting by artificially wounding canes and inoculating with *F. avenaceum*. Sporulation and necrosis after artificial inoculation of field grown primocane pieces in the in vitro test were significantly correlated with data collected after natural infection in the field (Fig. 4), demonstrating the applicability of this test and supporting the speculation that *F. avenaceum* is the primary causal pathogen. We observed that infection success of *F. avenaceum* depends on wounds (openings), thus supporting the observation that natural openings (e.g. split canes) facilitate natural infection with *F. avenaceum* in the field (Woodhead et al. 2013). Some cultivars (e.g. 'Tayberry', 'Buckingham Tayberry' or 'Valentina') allowed very little to no sporulation of *F. avenaceum* in the in vitro assay (Fig. 3), indicating that a mechanism of quantitative disease resistance might stop propagation of *F. avenaceum* in those cultivars even if this first barrier has been surpassed by wounding or splitting of canes in the field.

The highest degree of resistance in vitro as well as in the field was found in the *Rubus* hybrid cultivars

Table 3 List of all 36 *Rubus* genotypes used to assess ploidy levels

Cultivar	Pedigree	Ploidy
Raspberry		
Aroma Queen	Autumn Bliss open pollinated	2n = 2x
Autumn Bliss	Complex parentage includes Lloyd George, <i>R. ideaus strigosus</i> , <i>R. arcticus</i> , <i>R. occidentalis</i>	2n = 2x
Black Jewel	(Bristol × Dundee) × Dundee	2n = 2x
Dorman Red	<i>Rubus parvifolius</i> L. × Dorsett	2n = 2x
Rafzmach/Elida®	Malling Admiral × Chilcotin	2n = 2x
Gelbe Antwerpener	Unknown	2n = 2x
Glen Ample	Derivative of G. Prosen, Meeker, Preussen, M. Promise ‘SCRI 7326E1’ × ‘SCRI 7412H’	2n = 2x
Glen Coe	‘SCRI 7751C4’ × Glen Prosen	2n = 2x
Gradina	Malling Exploit × Rubin	2n = 2x
Rafzelsa/Himbostar®	Rote Wädenswiler open pollinated	2n = 2x
Kozachka	Blagorodna × Shtambovyi-19	2n = 2x
Resa/Lucana®	Unknown	2n = 2x
Lumina	Unknown	2n = 2x
Malahat	Meeker × BC/SCRI 7853/116	2n = 2x
Malling Promise	Newburgh × (Lloyd George × Pyne’s Royal)	2n = 2x
Meeker	Willamette × Cuthbert	2n = 2x
Oktavia	Malling Hestia × Glen Ample	2n = 2x
Polana	Heritage × Zeva Herbsternte	2n = 2x
Polesie		2n = 2x
Polka		2n = 2x
Radziejowa		2n = 2x
Malling Minerva	Complex parentage including M. Promise, <i>R. crataegifolius</i> , <i>R. occidentalis</i> , <i>R. idaeus strigosus</i> × SCRI 7269/67	2n = 2x
Royalty	N.Y. 252 × N.Y. 17861	2n = 2x
Rubaca/Niniane®	Rutrigo × Latham	2n = 2x
Rumla	Unknown	2n = 2x
Saxa Record	Autumn Bliss open pollinated	2n = 2x
Schönemann	Lloyd George × Preußen	2n = 2x
Tarusa	Shtambovyi-1 × Stolichnaya	2n = 2x
Tula Magic	Autumn Bliss × Tulameen	2n = 2x
Tulameen	Nootka × Glen Prosen	2n = 2x
Wei-Rula	Rutrigo × Latham	2n = 2x
Willamette	Newburgh × Lloyd George	2n = 2x
Zoltij Gigant	Maroseika × Ivanovskaya	2n = 2x
Other <i>Rubus</i> species		
Buckingham Tayberry	Spine-free sports of ‘Tayberry’	2n = 6x
Navaho	Ark. 583 (Thornfree × Brazos) × Ark. 631 (Ark. 550 × Cherokee)	2n = 4x
Tayberry	Aurora (2n = 8x + 2 = 58) × SCRI 626/67 (2n = 4x = 28)	2n = 6x

‘Tayberry’, ‘Buckingham Tayberry’ and ‘Dorman Red’. Hence, these three cultivars might be well suited for future breeding programs. However, both ‘Tayberry’ types are hybrid offspring of the blackberry ‘Aurora’ ($2n = 8x + 2 = 58$) and the tetraploid raspberry breeding clone ‘SCRI 626/67’ (Thompson 1995). Moreover, ‘Dorman Red’ is a hybrid of *R. idaeus* cv. ‘Dorsett’ ($2n = 2x = 14$) and *R. parvifolius* ($2n = 2x = 14$ or $2n = 4x = 28$). Because inter-ploidy crosses are often difficult and in order to avoid possible disturbances in future crosses (e.g. problems in chromosome pairing known for inter-ploidy crosses), we evaluated ploidy level of the *Rubus* genotypes available the Julius Kühn-Institut in Dresden. ‘Tayberry’ and ‘Buckingham Tayberry’ are hexaploid ($6 \times$) and the blackberry cultivar ‘Navaho’ is tetraploid ($4 \times$) (Table 3), (Meng and Finn 2002; Thompson 1995). However, the *Rubus* hybrid cultivar ‘Dorman Red’ was found to be diploid. Thus, it provides a valuable source of resistance introgression into red raspberry (*R. idaeus*) germplasm. Other sources of resistance, which have not been tested in this study might be found in wild *R. idaeus* germplasm (Hall et al. 2009). As of now, the genetic architecture of the field resistance displayed in the above cultivars is unknown. Several studies have identified sources of field resistance against multiple pathogens in numerous other plant species which can be contributed by individual major effect QTL or multiple minor effect QTL (Wiesner-Hanks and Nelson 2016).

In general, future breeding programs for resistance to cane diseases in Germany should encompass a combined strategy. Diploid germplasm of related, intercrossable *Rubus* species (e.g. ‘Dorman Red’) or wild *R. idaeus* accessions should be used to widen the gene pool of raspberries. Moreover, breeding programs employing marker assisted selection should focus on breeding of cultivars with little tendency towards cane splitting (Woodhead et al. 2013), resistance towards cane midge and towards *F. avenaceum* upon artificial (in vitro) inoculation. Steadily decreasing costs of genomic sequencing make it feasible to map quantitative trait loci (QTL) by association mapping in germplasm collections and conventional QTL mapping in suitable bi-parental mapping populations. Using these techniques, QTLs for resistance towards cane midge and resistance towards *F. avenaceum* after wounding and artificial

(in vitro) inoculation could be found and molecular markers derived for breeding purposes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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