Resistance of *Vitis* Germplasm to *Elsinoë ampelina* (de Bary) Shear Evaluated by Lesion Number and Diameter

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Abstract. We evaluated the resistance of 133 grapevine cultivars or selections, including *Vitis vinifera* and American hybrids, on the basis of lesion number and length to identify sources of resistance to grapevine anthracnose. All germplasms tested in this study showed anthracnose symptoms to some extent, and the distribution of lesion number and diameter was continuous. Most table grape *V. vinifera* cultivars were highly susceptible, showing many large lesions. However, *V. vinifera* wine grapes were more resistant with smaller lesions. Some American hybrid grapes such as ‘Ontario’ showed very few and small lesions. There was a significant positive correlation between lesion number and size in American ($r = 0.63$, $P = 0.0041$) and Japanese hybrids ($r = 0.56$, $P < 0.001$), whereas there was no correlation between these characters in *V. vinifera*. Japanese tetraploid cultivars were neither highly susceptible nor resistant. High anthracnose susceptibility of most well-known table grape *V. vinifera* cultivars, including ‘Muscat of Alexandria’, ‘Italia’, ‘Rizamat’, ‘Kattakurgan’, and ‘Thompson Seedless’, indicates that resistance should be introgressed from other cultivars such as American hybrids or wine grapes when these susceptible table grapes or their descendants are used in breeding anthracnose-resistant table grapes.

Grapevine anthracnose, caused by the ascomycete *Elsinoë ampelina* (de Bary) Shear (Shear, 1929), is one of the major diseases in humid regions. The disease is of European origin (Mirica, 1994; Shear, 1929) but has spread worldwide (Magarey et al., 1993; Mirica, 1994). In Japan, fungicides are applied six to eight times from April to June to control diseases, including anthracnose. To lessen the risk of the damage to the crop and to reduce the application of fungicide, cultivars resistant to this disease are indispensable.

Anthracnose resistance of grapevine cultivars has been reported (Fennell, 1948; Kawakami, 1932; Mortensen, 1981; Winkler, 1962; Yun et al., 2006), and it is consistent in these literatures that *V. labrusca* and some of its hybrids with *V. vinifera*, released mostly

in the United States, confer some resistance to the disease. These hybrid cultivars have long been produced in Japan since their introduction at the end of the 19th century because of their disease resistance and tolerance to berry cracking. Tetraploid hybrid cultivars have been developed, and their production is now increasing with the aid of the innovative application of plant growth regulators such as gibberellic acid to young clusters and berries to induce seedlessness and berry enlargement.

V. vinifera cultivars have usually been rated as susceptible to anthracnose; however, some are indicated to confer some resistance (Fennell, 1948; Kawakami, 1932; Winkler, 1962). Fennell (1948) rated the anthracnose resistance of *V. labrusca* as 8 and that of *V. vinifera* as 0 to 3 on a scale of 0 to 10, where 0 indicated completely susceptible and 10 indicated completely resistant. Winkler (1962) reported that most, but not all, *V. vinifera* cultivars were highly susceptible to anthracnose. However, they did not describe in detail the differences in resistance among cultivars. In Japan, Kawakami (1932) described the anthracnose resistance of *V. vinifera* cultivars, classifying ‘Pinot’, ‘Malaga’, ‘Riesling’, and

‘Muscat Hamburg’ as resistant and ‘Thompson Seedless’, ‘Muscat of Alexandria’, and ‘Flame Tokay’ as susceptible cultivars.

Mortensen (1981) recorded the anthracnose symptom severity of 67 *Vitis* clones for 4 to 6 years. These cultivars were classified on a scale of 1 to 7 (no symptoms to very severe symptoms), and some American hybrid cultivars, including ‘Blue Lake’, ‘Caco’, ‘Champanel’, ‘Concord’, ‘Delaware’, ‘Liberty’, ‘Ontario’, and ‘Urbana’, were evaluated as good sources of resistance. None of the *V. vinifera* cultivars tested were considered resistant, although the author mentioned that *V. vinifera* cultivars, including ‘Gulabi’ (synonym of ‘Muscat Hamburg’; Gurne and Kore, 1977), ‘Golden City’, and ‘Jakaranda’ (descendants of ‘Koenigin der Weingaerten’ × ‘Pearl of Csaba’) had previously been reported as resistant (Evans, 1971). Yun et al. (2006) evaluated 61 cultivars by scoring the numbers of lesions on leaves in a greenhouse after artificial inoculation and that of the lesions in a vineyard by natural infection. On the basis of artificial inoculation, the *V. vinifera* cultivars Black Swan, Rosario Bianco, and Kaiji were rated as susceptible, whereas the hybrid tetraploid cultivars Kyoho and Benifuji were moderately susceptible. The American hybrid cultivars, Campbell Early, Niagara, Sheridan, and Izumo Queen were rated as resistant.

Macroscopic rating based on foliar symptoms has been a common evaluation method throughout these studies. This method is straightforward and is appropriate for evaluating the resistance of the cultivars in the field. However, the method is costly and inefficient in terms of land use given that grape is a perennial vine crop. We have previously shown that evaluations based on lesion number and length can be reliable for rating the resistance to anthracnose using greenhouse-grown cuttings (Kono et al., 2012). The objective of this study was to evaluate anthracnose resistance using 39 *V. vinifera* cultivars or selections, 19 American hybrid cultivars, 49 Japanese hybrid selections, 17 tetraploid cultivars, and nine wild-type species or other cultivars of different origin based on lesion number and length. We aimed to compare anthracnose resistance within and between these groups and to identify resistant genetic resources for breeding.

Materials and Methods

Preparation of cuttings for detached-leaf assay. Rooted cuttings of total 133 cultivars or selections were prepared at the National Institute of Fruit Tree Science vineyards. We evaluated 35, 66, and 78 cultivars or selections in 2009, 2010, and 2011, respectively, as shown in Table 1. Cuttings with one bud were planted in a 72-cell tray (cells 4 cm × 4 cm × 5 cm; Takii & Co., Kyoto, Japan) with peatmoss-based soil (BM1; Berger Peat Moss, Saint-Modeste, Quebec, Canada) from late February to early March from 2009 to 2011. The cuttings were discarded each year after the experiment, and new cuttings were prepared in the following year. A mat-shaped

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Table 1. Anthracnose resistance of grape germplasms tested from 2009 to 2011.

Accession name	Ploidy	Species	Group	Origin ²	Mean lesion diam. (mm)				Mean lesion number				Estimated lesion area (mm ²)
					2009	2010	2011	Mean	2009	2010	2011	Mean	
126-15	2	Hybrid	JH	Japan			2.3	2.3			53	53	213
134-96	2	Hybrid	JH	Japan			2.3	2.3			32	32	135
168-35	2	Hybrid	JH	Japan			2.1	2.1			26	26	86
173-2	2	Hybrid	JH	Japan		3.3		3.3		50		50	418
177-48	2	Hybrid	JH	Japan			1.9	1.9			40	40	111
177-70	2	Hybrid	JH	Japan			1.5	1.5			32	32	56
384-15	4	Hybrid	TP	Japan			2.5	2.5			42	42	206
406-1	3	Hybrid	TP	Japan		2.7		2.7		43		43	246
608-8	2	Hybrid	JH	Japan		1.8		1.8		48		48	128
619-14	2	Hybrid	JH	Japan			2.9	2.9			65	65	430
620-2	2	Hybrid	JH	Japan		2.0		2.0		35		35	110
628-22	2	Hybrid	JH	Japan			2.6	2.6			14	14	70
628-95	2	Hybrid	JH	Japan			2.1	2.1			44	44	154
629-16	2	Hybrid	JH	Japan			3.0	3.0			62	62	440
632-15	2	Hybrid	JH	Japan		1.8		1.8		24		24	60
634-21	2	Hybrid	JH	Japan			2.7	2.7			48	48	275
634-81	2	Hybrid	JH	Japan		3.5		3.5		51		51	496
638-46	2	Hybrid	JH	Japan			2.5	2.5			42	42	210
639-22	2	Hybrid	JH	Japan		1.2		1.2		27		27	33
640-47	2	Hybrid	JH	Japan		2.9		2.9		48		48	316
643-66	2	Hybrid	JH	Japan			1.8	1.8			19	19	52
644-59	2	Hybrid	JH	Japan			2.9	2.9			45	45	304
645-23	2	<i>V. vinifera</i>	OV	Japan			2.8	2.8			45	45	283
645-30	2	<i>V. vinifera</i>	OV	Japan			2.4	2.4			27	27	125
645-73	2	<i>V. vinifera</i>	OV	Japan		3.2		3.2		36		36	286
651-6	2	Hybrid	JH	Japan		2.8		2.8		58		58	357
655-1	2	Hybrid	JH	Japan			1.4	1.4			39	39	63
656-22	2	Hybrid	JH	Japan			2.7	2.7			50	50	290
658-9	2	Hybrid	JH	Japan		2.9		2.9		40		40	271
661-23	2	Hybrid	JH	Japan			2.6	2.6			42	42	215
662-58	2	Hybrid	JH	Japan		2.7		2.7		54		54	302
662-6	2	Hybrid	JH	Japan		3.5		3.5		73		73	708
667-6	2	Hybrid	JH	Japan		3.1		3.1		53		53	395
668-12	2	Hybrid	JH	Japan			1.6	1.6			45	45	86
668-124	2	Hybrid	JH	Japan		1.3		1.3		32		32	42
668-2	2	Hybrid	JH	Japan		2.2		2.2		32		32	123
668-4	2	Hybrid	JH	Japan		2.4		2.4		35		35	162
669-1	2	Hybrid	JH	Japan		3.7		3.7		69		69	723
669-8	2	Hybrid	JH	Japan			2.8	2.8			63	63	381
675-100	2	Hybrid	JH	Japan			1.9	1.9			47	47	133
675-125	2	Hybrid	JH	Japan		3.3		3.3		45		45	383
675-75	2	Hybrid	JH	Japan			2.1	2.1			43	43	144
677-62	2	Hybrid	JH	Japan			2.0	2.0			33	33	105
707-33	2	Hybrid	JH	Japan			2.6	2.6			42	42	215
719-11	2	Hybrid	JH	Japan		2.8		2.8		54		54	341
Aki Queen	4	Hybrid	TP	Japan			2.3	2.3			28	28	119
Akitsu 20	4	Hybrid	TP	Japan			2.2	2.2			12	12	46
Akitsu 21	2	Hybrid	JH	Japan			2.9	2.9			21	21	134
Akitsu 28	4	Hybrid	TP	Japan			1.8	1.8			9	9	24
Akitsu 29	4	Hybrid	TP	Japan			2.3	2.3			33	33	140
Akitsu 30	4	Hybrid	TP	Japan			1.4	1.4			28	28	44
Akitsu 7	2	Hybrid	JH	Japan			1.7	1.7			23	23	54
Alphonse Lavallee	2	<i>V. vinifera</i>	OV	France	2.6	2.9		2.8	46	53		49	297
Buffalo	2	Hybrid	AH	USA		2.0		2.0		31		31	102
Cabernet Franc	2	<i>V. vinifera</i>	VW	France		2.8		2.8		28		28	168
Cabernet Sauvignon	2	<i>V. vinifera</i>	VW	France	2.4	1.7		2.0	47	30		39	128
Campbell Early	2	Hybrid	AH	USA			1.3	1.3			8	8	11
Cardinal	2	<i>V. vinifera</i>	OV	USA	2.8	2.6		2.7	46	51		49	275
Carignan	2	<i>V. vinifera</i>	OV	France	3.8			3.8	50			50	565
Chardonnay	2	<i>V. vinifera</i>	VW	France			0.6	0.6			40	40	12
Chasselas Blanc	2	<i>V. vinifera</i>	VW	Unknown		0.7	0.9	0.8		43	16	29	16
Concord ³	2	Hybrid	AH	USA	1.6	1.3	1.5	1.5	33	28	7	23	39
Dark Ridge	4	Hybrid	TP	Japan			2.1	2.1			19	19	66
Delaware ⁴	2	Hybrid	AH	USA	0.5	0.6	0.5	0.5	31	49	35	38	9
Exotic ⁵	2	<i>V. vinifera</i>	OV	USA	1.7	2.2	1.8	1.9	48	57	59	55	152
Flame Tokay	2	<i>V. vinifera</i>	OV	Algeria	2.6			2.6	45			45	241
Fujiminori	4	Hybrid	TP	Japan			1.6	1.6			38	38	75
Fukuoka 15	2	Hybrid	JH	Japan			2.0	2.0			64	64	200
Fukuoka 16	4	Hybrid	TP	Japan			2.2	2.2			30	30	119
Golden Muscat	2	Hybrid	AH	USA	2.6	2.5		2.6	37	42		39	203
Hakunan	2	<i>V. vinifera</i>	OV	Japan	3.5	3.3		3.4	66	64		65	594
Himrod	2	Hybrid	AH	USA	2.9	2.4		2.7	53	45		49	271
Honey Venus	4	Hybrid	TP	Japan	2.6		2.1	2.3	35		34	35	148

(Continued on next page)

Table 1. (Continued) Anthracnose resistance of grape germplasm tested from 2009 to 2011.

Accession name	Ploidy	Species	Group	Origin ²	Mean lesion diam. (mm)				Mean lesion number				Estimated lesion area (mm ²)
					2009	2010	2011	Mean	2009	2010	2011	Mean	
Iku 71	2	Hybrid	JH	Japan		2.2		2.2		32		32	120
Iku 82	2	Hybrid	JH	Japan			2.9	2.9			59	59	400
Italia ³	2	<i>V. vinifera</i>	OV	Italy	3.0	3.3	2.8	3.0	49	45	51	48	349
Katta Kurgan	2	<i>V. vinifera</i>	OV	Uzbekistan	3.6			3.6	47			47	470
Keuka	2	Hybrid	AH	USA	1.4	1.8		1.6	29	22		25	50
Kober 5 BB	2	Hybrid	Others	Austria			1.1	1.1			38	38	36
Koshu	2	Unknown	Others	Japan		1.2	0.8	1.0		22	13	17	13
Koshu Sanjaku	2	Unknown	Others	Japan			0.9	0.9			33	33	20
Kyoho ³	4	Hybrid	TP	Japan	2.3	2.5	2.2	2.4	44	42	18	35	152
Lake Emerald ³	2	Hybrid	AH	USA	1.7	2.3	1.2	1.8	16	13	3	11	26
Lemberger	2	<i>V. vinifera</i>	VW	Austria		3.0		3.0		59		59	414
Malaga	2	<i>V. vinifera</i>	OV	France	3.1	2.7		2.9	59	47		53	345
Merlot	2	<i>V. vinifera</i>	VW	France			2.2	2.2		59	49	54	197
Mission	2	<i>V. vinifera</i>	VW	Spain			2.2	2.2			41	41	161
Muller Thurgau	2	<i>V. vinifera</i>	VW	Germany			2.0	2.0			18	18	55
Muscat Hamburg	2	<i>V. vinifera</i>	OV	UK			1.7	1.7			46	46	109
Muscat of Alexandria ³	2	<i>V. vinifera</i>	OV	Italy	3.1	3.1	3.2	3.1	50	51	31	44	339
Neo Muscat	2	<i>V. vinifera</i>	OV	Japan			2.9	2.9			48	48	315
New York Muscat	2	Hybrid	AH	USA		2.8		2.8		57		57	342
Niagara	2	Hybrid	AH	USA		1.9		1.9		16		16	45
North Black	2	Hybrid	JH	Japan	2.2			2.2	35			35	129
Ontario ³	2	Hybrid	AH	USA	0.8	0.9	0.7	0.8	11	9	4	8	4
Oriental Star	2	Hybrid	JH	Japan			3.0	3.0			31	31	214
Perlette ³	2	<i>V. vinifera</i>	OV	USA	2.2	1.5	1.4	1.7	55	61	56	57	126
Pinot Blanc	2	<i>V. vinifera</i>	VW	France		1.7		1.7		39		39	88
Pinot Noir	2	<i>V. vinifera</i>	VW	France		1.8	1.2	1.5		49	26	37	63
Pione ³	4	Hybrid	TP	Japan	2.0	1.5	1.8	1.8	49	56	60	55	134
Pizzutello Bianco	2	<i>V. vinifera</i>	OV	Moldova		3.2		3.2		66		66	534
Pobeda	2	<i>V. vinifera</i>	OV	Uzbekistan			0.7	0.7			53	53	20
Portland	2	Hybrid	AH	USA		1.4		1.4		11		11	17
Queen Nina	4	Hybrid	TP	Japan			2.3	2.3			13	13	56
Reliance	2	Hybrid	AH	USA	2.4			2.4	41			41	183
Riesling	2	<i>V. vinifera</i>	VW	Germany			1.8	1.8			36	36	92
Rizamat	2	<i>V. vinifera</i>	OV	Uzbekistan		3.2	3.0	3.1		48	47	47	356
Rosario Bianco	2	<i>V. vinifera</i>	OV	Japan		3.2		3.2		64		64	531
Schuyler	2	Hybrid	AH	USA		1.5		1.5		14		14	26
Sekirei	2	<i>V. vinifera</i>	OV	Japan	3.6	3.4		3.5	62	38		50	483
Seneca	2	Hybrid	AH	USA	2.6	2.3		2.4	23	35		29	136
Seto Giants	2	<i>V. vinifera</i>	OV	Japan	2.2	2.2		2.2	59	56		58	220
Shine Muscat	2	Hybrid	JH	Japan	2.7			2.7	48			48	276
Shiraz	2	<i>V. vinifera</i>	VW	France		1.4	1.0	1.2		44	26	35	38
Stover	2	Hybrid	AH	USA		1.8		1.8		36		36	93
Sun Verde	4	Hybrid	TP	Japan			2.1	2.1			21	21	70
Sunny Rouge ³	4	Hybrid	TP	Japan	1.5	1.3	1.1	1.3	34	49	32	39	53
Thompson Seedless ³	2	<i>V. vinifera</i>	OV	Turkey	3.6	3.3	3.0	3.3	50	42	57	50	429
Urbana ³	2	Hybrid	AH	USA	1.8	0.8	1.1	1.2	37	23	16	25	29
<i>V. candicans</i> 'Leverkuhn'	2	<i>V. candicans</i>	Others	USA		2.3		2.3		48		48	196
<i>V. coriacea</i>	2	<i>V. coriacea</i>	Others	USA			1.2	1.2			9	9	11
<i>V. labrusca</i>	2	<i>V. labrusca</i>	Others	USA			0.8	0.8			6	6	3
<i>V. riparia</i> 'Gloire de Montpellier'	2	<i>V. riparia</i>	Others	USA			0.6	0.6			10	10	3
<i>V. riparia</i> spp.	2	<i>V. riparia</i>	Others	USA	0.3	0.2		0.3	54	53		53	3
<i>V. rupestris</i> 'Constantia'	2	<i>V. rupestris</i>	Others	South Africa		3.0		3.0		25		25	175
Van Buren	2	Hybrid	AH	USA	1.3	1.0		1.1	21	22		22	22
Wayne	2	Hybrid	AH	USA	1.8	1.2		1.5	23	12		17	29
Yamanashi 48	2	<i>V. vinifera</i>	VW	Japan			1.9	1.9			30	30	89
Yamanashi 49	2	<i>V. vinifera</i>	VW	Japan			1.2	1.2			52	52	55
Yamanashi 53	2	<i>V. vinifera</i>	VW	Japan			1.6	1.6			35	35	73
Yamanashi 54	2	Hybrid	JH	Japan			1.8	1.8			23	23	59
Yoho	4	Hybrid	TP	Japan	2.0			2.0	25			25	81
Zweigeltrebe	2	<i>V. vinifera</i>	VW	Austria			1.7	1.7			37	37	80

LSD⁴

0.53 0.55 0.49 14 18 22

²Information about the country of origin is based on the Vitis International Variety Catalogue (VIVC: <<http://www.vivc.de/>>), except for cultivar/selections of Japanese origin.³The data of these cultivars were used in a previous study (Kono et al., 2012).⁴LSD = Fisher's protected least significant difference test at $P < 0.05$.VW = *V. vinifera* wine grapes; OV = other *V. vinifera* grapes; AH = American hybrids; JH = Japanese hybrid cultivars and selections; TP = tetraploids.

heater with a thermostat was placed under the tray to maintain the soil temperature at 25 °C and to enhance root formation. The greenhouse was maintained at an air temperature of

less than 30 °C using an air conditioner to avoid temperature stress on the cuttings. After two or three leaves had fully expanded and roots had emerged, cuttings were planted

individually in 12-cm pots containing commercial soil (nitrogen at 200 mg·L⁻¹, phosphorus at 2000 mg·L⁻¹, and potassium at 200 mg·L⁻¹; JA-no-tsuchi, Zen-Noh, Tokyo,

Japan). Plants were grown in the same greenhouse until they were used for the experiment. To prevent the growth of pathogens or other microbes in water droplets on the leaves, plants were watered from the bottom of pots. A plastic-coated iron stake (70 cm in length) was used as a support for each potted plant, and plants were pinched routinely at the top of this prop to obtain new shoots. Leaves were sampled from up to three healthy cuttings of each cultivar.

Inoculation of detached leaves with *E. ampelina*. The detached leaves were inoculated with *E. ampelina* conidia as previously described (Kono et al., 2009) with some modifications. The *E. ampelina* isolate Akitsu01 was used for all inoculations. Healthy expanding leaves were inoculated because young leaves are most susceptible to infection (Mirica, 1994). The sampled leaf blades were 3 to 6 cm long. A healthy expanding leaf was sampled from a cutting weekly or once every 2 weeks, and these detached leaves were inoculated. These leaves corresponded to the second or third leaf from the shoot apex. Detached leaves were incubated on 0.5% (w/v) agar plates with the petioles embedded in the agar. Two $\approx 1\text{-cm}^2$ pieces of gauze were placed on each leaf, and 25 μL of conidial suspension (1×10^3 and 5×10^3 conidia/mL, respectively) was applied to each piece. Petri dishes were covered and wrapped with polyethylene bags (0.03 mm thickness) to maintain greater than 95% relative humidity and were then incubated at 27 °C under continuous light. The gauze and polyethylene bags were removed after 2 d, and the leaves were further incubated under the same conditions for 14 d in total with the lids of the petri dishes closed to prevent the leaves from wilting.

Measurement of lesion number and length on detached leaves. Nine inoculated detached leaves per cultivar were used to measure lesion number and length. To measure both parameters, inoculated leaves that showed at least one lesion after inoculation were studied, and pinpoint lesions were included for measurement of lesion number and length. The number of lesions was recorded at the site that had been inoculated with 5×10^3 conidia/mL with the purpose of comparing the lesion number of each cultivar under the same inoculation pressure. The number was recorded 5 to 7 d postinoculation (dpi) before the lesions coalesced. To measure lesion length, we took photographs (2848×2136 pixels) of the inoculated leaves at 14 dpi to measure the diameter of the lesions. Because large lesions coalesced at 14 dpi at the site that had been inoculated with 5×10^3 conidia/mL, we measured lesion diameter at the site that had been inoculated with 1×10^3 conidia/mL. In resistant cultivars, because they showed few colonies at the site that had been inoculated with 1×10^3 conidia/mL, we measured lesion diameter at both inoculated sites. The photograph was then magnified digitally at approximately $\times 10$ to allow more precise measurement of lesions. A ruler was photographed as a scale bar beside the lesions in the same focal plane. For any lesion that was

elliptical or irregularly shaped, the length of the major axis was measured. Any merged lesions with an individual length that could not be measured were omitted. On each sampled leaf, all discernible lesions (up to 20) were measured, and the average lesion length was calculated.

Grouping the cultivars. To compare the variation of resistance between cultivars or selections with different genetic backgrounds, we grouped all the cultivars or selections tested into six classes: *V. vinifera* wine grapes (VW), other *V. vinifera* grapes (OV), American hybrids (AH), Japanese hybrid cultivars and selections (JH), tetraploids (TP), and others. All the VW and OV have only *V. vinifera* origin; we defined VW as cultivars or selections belonging to western European grape (G3 to G12) by Aradhya et al. (2003) based on microsatellite analysis or their relatives. All VW were wine grapes except for ‘Chasselas Blanc’, which is also consumed as a table grape. OV consists of central European grapes and Mediterranean table grapes (Aradhya et al., 2003) or their descendants, and most of them are table grapes, whereas the wine grape ‘Carignan’ was included in this group because it is classified as a central European grape (G13). We defined AH as cultivars released in the United States having *V. labrusca* or other American wild species origin. JH and TP included cultivars or selections hybridized in Japan using AH and *V. vinifera*. TP included one triploid selection (406-1). We grouped other cultivars of uncertain origin, rootstocks, or wild species into “other.” ‘Koshu’ and ‘Koshu Sanjaku’ are thought to be Japanese native *V. vinifera* cultivars; however, their phenetic distance from other *V. vinifera*, including Chinese *vinifera* cultivars, based on microsatellite analysis was high (Goto-Yamamoto et al., 2006). Furthermore, at the VVS2 locus (Goto-Yamamoto et al., 2009), ‘Koshu’ harbors an allele that is uncommon among *V. vinifera* and may be a hybrid between *V. vinifera* and Asian native *Vitis* species (Goto-Yamamoto, personal communication). Therefore, we categorized these two as of uncertain origin. Because “other” comprised clones with various genetic backgrounds, we did not use these entries for further comparison with the other groups.

Statistical analysis. Data were analyzed using R software (Version 2.15.0; R Development Core Team, 2012). In each year, the cultivars’ means were compared using Fisher’s protected least significance difference value based on the analysis of variance in a completely randomized design with nine leaf replicates with significance at $P < 0.05$. Broad-sense heritability estimates for lesion number and diameter were as high as 0.75 and 0.88, respectively, for 1 year of data (Kono et al., 2012). Because broad-sense heritability (h^2) was calculated using the equation $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2)$, where σ_E^2 is the environmental variance and σ_G^2 is the genetic variance of the individual (Yamada et al., 1993), 75% (lesion number) and 88% (lesion diameter) of variation within the 1-year evaluation

originate from genetic variance. Therefore, we considered these data to be sufficiently reliable for resistance evaluation in the cultivars and selections. When multiple years of evaluation were available, grand means of lesion number and size based on 2- or 3-year evaluation were calculated and were used for subsequent analysis. Estimated lesion area (EA) was calculated on the basis of the grand means of lesion number (N) and diameter (D) using the equation $EA = N \times (D/2)^2 \times \pi$.

To compare the distributions of the five groups (e.g., VW, OV, AH, JH, and TP), box plots based on number, diameter, and EA were constructed (Fig. 1). Notches are shown in each box, and failure of the notches of two plots to overlap provides strong evidence that the two medians differ (Chambers et al., 1983). For each group of genotypes we calculated the Pearson product-moment correlation coefficient between lesion number and diameter (Fig. 2).

Fig. 1. Distribution of mean lesion number (A), mean lesion diameter (B), and estimated lesion area (C) among five different *Vitis* groups. Small dots are outliers in each group. If the notches of two plots do not overlap, this is strong evidence that the two medians differ (Chambers et al., 1983).

Fig. 2. Correlation between mean lesion diameter and number in different *Vitis* groups. (A) All the genotypes tested, including *V. vinifera* wine grapes (VW: triangle), other *V. vinifera* grapes (OV: closed circle), American hybrids (AH: x), Japanese hybrid cultivars and selections (JH: +), tetraploids (TP: diamond). (B) OV. (C) VW. (D) AH. (E) JH. (F) TP. NS, **, *** Nonsignificant or significant at $P \leq 0.01$ and 0.001 , respectively.

Results and Discussion

Anthracoze resistance among all the cultivars and selections tested. We found no germplasm that was immune (completely resistant) to anthracnose. The distribution of lesion number and diameter was continuous (Table 1). This result is consistent with those of previous studies (Mortensen, 1981; Yun et al., 2006) and suggests that the prevailing resistance genes among grapevine cultivars and selections are quantitative.

To compare the variation of resistance between cultivars or selections with different parentage, we evaluated the distribution between five different groups, VW, OV, AH,

JH, and TP (Fig. 1). OV showed a large number of lesions among the groups tested (Fig. 1A). In contrast, the lesion number of VW was lower than that of OV, with a significant difference between their medians. We observed a wider range of variation in AH and TP, but they also showed fewer lesions than OV. JH showed the widest distribution. The distribution of lesion diameter was almost the same as that of lesion number (Fig. 1B). VW showed a far narrower distribution than OV and JH. Lesion diameters of AH, TP, and VW were similar, but TP showed larger lesions with smaller variation than the other two groups. EA of OV and JH was significantly larger than that of VW, TP,

and AH, and these three groups showed similar EA (Fig. 1C). Because EA is calculated from lesion number and diameter, the correlations calculated from the data of all clones between area and lesion number ($r = 0.77$, $P < 0.001$) or lesion diameter ($r = 0.88$, $P < 0.001$) were high. Although EA would be the best indicator for resistance estimated from lesion number and size, it is not an independent parameter and is dependent on lesion number and diameter. Therefore, below we have discussed in detail the resistance evaluated by lesion number or diameter.

First, we consider the relationship between the two independent parameters lesion number and size. We found a weak but significant correlation between the mean lesion number and diameter (Fig. 2A; $r = 0.52$, $P < 0.001$) using all the genotypes tested. Because R^2 is 0.27, approximately one-fourth of the variation in lesion number evaluation could be predicted by the regression on lesion diameter and vice versa. We found a consistent correlation between lesion number and size in AH (Fig. 2D; $r = 0.63$, $P = 0.0041$) and JH (Fig. 2E; $r = 0.56$, $P < 0.001$). This finding indicates that a hybrid cultivar with small lesions tends to show fewer lesions than that with large lesions and that we could partially predict lesion number from lesion diameter in AH and JH. Because lesion diameter is a more reliable trait, with higher broad-sense heritability than lesion size as previously discussed (Kono et al., 2012), lesion diameter was used as a reliable indicator for resistance prediction in this study. In contrast, OV (Fig. 2B; $r = 0.0043$, $P = 0.98$), VW (Fig. 2C; $r = 0.19$, $P = 0.48$), and TP ($r = -0.028$, $P = 0.92$) did not show significant correlations (Fig. 2). Intercepts of OV (50) and VW (33) were significant and high, but neither of the slopes was significant, suggesting that *V. vinifera* cultivars show many lesions despite their lesion size. The slope or intercept of TP was not significant, indicating no relationship between lesion number and diameter among these cultivars. Next, we discuss in detail the differences in resistance between cultivars in each group.

Anthracoze resistance in *V. vinifera*. OV showed more and larger lesions than that in VW, but *V. vinifera* showed surprising variation in average lesion diameter, ranging from 0.6 ('Chardonnay') and 0.7 ('Pobeda') mm to 3.8 ('Carignan') and 3.6 mm ('Katta Kurgan'). Most of the highly susceptible cultivars, showing lesions greater than 3 mm on average, were table grapes (Table 1). Some of these, 'Muscat of Alexandria' (Fig. 3), 'Italia', 'Rizamat', 'Kattakurgan', and 'Thompson Seedless', are well-known table grape cultivars and are also important genetic resources for table grape breeding. They also showed many (greater than 44) lesions and are therefore considered to be highly susceptible to anthracnose. For anthracnose-resistant table grape breeding, susceptibility traits of these cultivars may be a serious concern. Therefore, introgression of anthracnose resistance should be from cultivars of other origin.

Fig. 3. Typical lesions on 'Muscat of Alexandria' (left) and 'Chasselas Blanc' (right) at 14 d post-inoculation (dpi). Bar = 5 mm.

Several table grape cultivars, including 'Perlette', 'Exotic', 'Muscat Hamburg', and 'Pobeda', showed medium or very small lesions, although their lesion numbers were 46 or greater. The male parent of 'Perlette' is 'Thompson Seedless', which showed high susceptibility. This result suggests that the moderate resistance evaluated by lesion diameter could be transmitted to the next generation even by hybridization using 'Thompson Seedless'. One of the parents of 'Perlette' is 'Koenigin der Weingaerten', which should have three-eighths of 'Chasselas Blanc' genome. Furthermore, because 'Chasselas Blanc' showed small lesions (0.8 mm; Fig. 3), 'Koenigin der Weingaerten' may have some resistance inherited from 'Chasselas Blanc', in consistency with the report that 'Golden City' and 'Jakaranda', descendants of 'Koenigin der Weingaerten', are resistant to anthracnose (Evans, 1971). Lesion diameter of 'Muscat Hamburg' was also intermediate, and one of its descendants, 'Pobeda', clearly showed a high level of resistance to anthracnose, evaluated by lesion diameter. The resistance of 'Sabalkanskoi', which is one of the parents of 'Pobeda', was not evaluated, preventing the identification of the parent giving this remarkable resistance. These results suggest that moderate resistance evaluated by lesion diameter could be achieved by accumulation of small-effect resistance genes even within *V. vinifera* parentage.

However, most VW exhibited clear resistance to anthracnose as evaluated by lesion diameter, and their parentages were different from OV. Among these wine grapes, 'Chardonnay' showed very small (0.6 mm) lesions. Four descendants of 'Chardonnay', 'Yamanashi 48, 49, 53, and 54 from a Japanese breeding program, also showed intermediate-sized (1.2 to 1.9 mm) lesions, although their resistance was not as high as that of 'Chardonnay'. The lesion diameters of 'Shiraz' (1.2 mm), 'Pinot Noir' (1.5 mm), and its mutant 'Pinot Blanc' (1.7 mm) were also small. This result suggests that there may be larger effect resistance genes and/or many more small effect resistance genes within wine grape populations than that within table grape populations. Because anthracnose is of European origin (Shear, 1929), and historical wine grapes, *occidentalis*, were developed in Europe, ancestral species of *V. vinifera* wine grapes may have acquired resistance to anthracnose from native *Vitis* species during the

long period of domestication in Europe, and their descendants may have been selected during culture based on anthracnose resistance and on wine quality. A hypothesis that the *occidentalis* are closer to the wild-type grape and possess many wild-type characteristics is supported by the fact that they share a cluster with wild-type grapevine *V. vinifera* ssp. *sylvestris*, as demonstrated in an analysis of eight microsatellite loci (Aradhya et al., 2003). Myles et al. (2011) also showed the possible origin of *vinifera* using a 9000-single nucleotide polymorphism genotyping array in the Near East with subsequent introgression from wild-type *sylvestris* into *V. vinifera* in Europe. However, old and important table grape cultivars may have had a different origin, supported by microsatellite analysis (Aradhya et al., 2003). In the areas where these table grapes were developed, anthracnose resistance may not have been an important trait for selection owing to the absence of pathogenic fungi or unfavorable environmental conditions for anthracnose.

AH and JH. The distribution of lesion number and size in AH were clearly different from those in OV (Fig. 1). Some hybrids showed significantly less lesions than *V. vinifera*, as discussed previously, suggesting that they harbor different resistance genes that prevent anthracnose from invading leaf tissues. For example, 'Ontario', 'Campbell Early', 'Portland', and 'Lake Emerald' showed few lesions, less than 11 on average, whereas all *V. vinifera* cultivars showed more lesions. Therefore, these varieties may be important parents for resistance breeding against anthracnose, particularly for introgressing resistance evaluated by lesion number. The reason for the resistance is unknown, but thick leaf hairs on young leaves may form a structural barrier to anthracnose infection. Lesion number and diameter were smaller than those of *V. vinifera*, on average. In particular, 'Delaware' and 'Ontario' showed very small lesions, 0.6 and 0.8 mm, respectively, as previously found (Kono et al., 2012).

Most of the JH were selected from the hybridization of AH and *V. vinifera* table grapes or were intercrosses of such selections. Therefore, these vines had more of *V. vinifera* table grape genome than the parent AH. Because 'Rizamat', 'Italia', and 'Katta Kurgan' have frequently been used in Japanese breeding programs and are highly susceptible to anthracnose, these cultivars may be the source of susceptibility. This hypothesis is consistent with our findings that the distribution of resistance observed in JH tended toward higher susceptibility than that in AH (Figs. 1 and 2) and that some selections were as susceptible as *V. vinifera* table grape cultivars. However, some Japanese selections were still highly resistant such as 639-22, 643-66, and 668-124 compared with *V. vinifera* parents. These selections should be used as parents to improve table grape breeding populations with respect to anthracnose resistance.

Tetraploids. Lesion diameter of tetraploid cultivars and selections ranged from 1.4 to

2.7 mm, suggesting that none of them was highly susceptible or highly resistant. In particular, 'Pione' and its descendants 'Sunny Rouge', 'Fujiminori', and 'Akitsu 30' showed relatively small lesions. Lesion numbers in TP distribution were also skewed to the lower side compared with *V. vinifera* (Fig. 1).

All the TP tested in this study had some 'Kyoho' origin. One of the parents of 'Kyoho' is a tetraploid bud sport of 'Campbell Early' ('Ishiharawase'), and 'Campbell Early' showed relatively small and few lesions (Table 1). Some resistance genes in 'Campbell Early' may have contributed to the resistance of 'Kyoho' and probably also of other tetraploid cultivars in Japan.

Wild-type species and other cultivars of uncertain origin. *V. rupestris* 'Constantia' showed lesions as large (3.0 mm) as *V. vinifera* table grape susceptible cultivars, although there were fewer lesions. However, other wild-type species showed small lesions (0.3 to 1.2 mm), and lesion numbers were also few, except for one *V. riparia* clone (53 lesions). Fruity and aromatic flavors of *V. labrusca* have been considerably used in the northeastern United States and Asia (Reisch et al., 2012); therefore, it is the most important wild species of AH. Moreover, we evaluated one *V. labrusca* clone showing very small and few lesions (0.8 mm, six lesions). This is consistent with our finding that some AH tested in this study showed some resistance, evaluated by both lesion diameter and number.

Resistance of Chinese *Vitis* species to anthracnose has been evaluated using 13 species. Five taxonomically undescribed grapes native to China based on the estimated percentage lesion covered the whole leaf area under no-spray conditions (Wang et al., 1998). All Chinese wild-type species were rated as resistant or highly resistant, although no "extremely resistant" clones showing no symptoms were found. In contrast, Mortensen (1981) found some species showing no symptoms such as *V. rotundifolia* and *V. munsoniana*. Because major resistance quantitative trait loci (QTL) genes for grapevine downy mildew such as *Rpv1* (Merdinoglu et al., 2003) and powdery mildew such as *Run1* (Pauquet et al., 2001) and *Ren4* (Ramming et al., 2011) originated in wild-type species, highly resistant wild-type species described in this study could be sources of major anthracnose resistance QTL. Our next task is to identify candidate resistance genes from such resistant genetic resources for sources of dominant qualitative resistance.

'Koshu' is a historical Japanese original cultivar known from the 12th century (Kawase, 1996) and has been shown to be closely related to 'Koshu Sanjaku', another old Japanese cultivar (Goto-Yamamoto et al., 2006) by microsatellite analysis. They showed very small lesions with low to intermediate lesion numbers, a level of resistance similar to that of 'Chasselas Blanc'. One of the reasons for the centuries-long survival of these cultivars in Japan may be anthracnose resistance.

Most of the important historical *V. vinifera* table grapes are highly susceptible to anthracnose, and we have found no genetic resource showing perfect resistance (immunity). Although it remains necessary to identify genetic resources that could be a source of dominant high resistance to this disease, we should combine small-effect resistance genes among available genetic resources to breed anthracnose resistance table grape cultivars. AH and some *V. vinifera* cultivars, such as 'Chasselas Blanc', could be used as resistance sources.

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