Identification of Resistance to the Large Raspberry Aphid in Black Raspberry

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ABSTRACT. The large raspberry aphid (*Amphorophora agathonica* Hottes) is an important vector of viruses in *Rubus* L. across North America. Although breeding for aphid resistance has long been recognized as an important tool for protecting red raspberries (*Rubus idaeus* L.) from viral infection, this is the first report of resistance to *A. agathonica* in black raspberry (*Rubus occidentalis* L.). Seedlings from 132 wild populations of black raspberries, representing the species' native range, were screened for resistance to *A. agathonica*. Strong resistance was found in three of these populations, one from Ontario (ORUS 3778), one from Maine (ORUS 3817), and one from Michigan (ORUS 4109). Resistance to the large raspberry aphid in ORUS 3778 and ORUS 3817 is dominant and appears to be conferred by different genes. We propose that the genes for resistance in ORUS 3778 and ORUS 3817 be designated *Ag₄* and *Ag₅*, respectively. Resistance to *A. agathonica* in ORUS 4109 also appears to be controlled by a dominant allele at a single locus, but cannot be differentiated from *Ag₄* at this time.

The black raspberry, often called “blackcap,” was first domesticated in the 1830s. A member of the Rosaceae, it is diploid ($2n = 2x = 14$) and belongs to the same subgenus (*Idaeobatus*) as the red raspberry, with which it readily crosses. Native to eastern North America from New Brunswick to the Carolinas and as far west as Kansas and Nebraska, black raspberries typically are found in disturbed habitats and near forest edges. The black raspberry industry in North America has undergone a slow but steady contraction since the 1920s due in large part to disease and a lack of adapted and resistant cultivars. Today, growers in Oregon, the leading production region, typically see a decline in production after the second harvest and they remove fields after only three to four seasons because of decreased profitability (Halgren et al., 2007). Much of this decline has been associated with aphid-vectored viruses, particularly black raspberry necrosis virus [BRNV (Halgren et al., 2007)], with disease pressure so high that fields often become 100% infected within a couple of growing seasons (Halgren, 2006).

Breeders have long recognized the importance of aphid resistance in the development of new raspberry cultivars that protect against virus infection (Schwartz and Huber, 1937). In Europe, at least 13 genes for resistance to the aphid *Amphorophora idaei* Börner (Homoptera: Aphididae) are known (Hall et al., 2009), and five biotypes of the aphid have been described (Hall et al., 2009; McMenemy et al., 2009). Resistance to *A. idaei* is derived from a variety of sources, including red and black raspberry. Keep (1989) indicated that the use of these resistance genes has been very effective in slowing the spread of aphid-vectored viruses in red raspberry at East Malling, UK. At the Scottish Crop Research Institute (Invergowrie), virus infection rates in plots of resistant cultivars was less than 10% after 3 years, while 100% of susceptible plants became infected during the same period (Jones, 1976).

In North America, the primary vector of the raspberry mosaic virus complex is the large raspberry aphid. Resistance to *Amphorophora agathonica* is conferred by three genes, all from red raspberry. The single dominant gene, *Ag₁*, is derived from the old UK cultivar Lloyd George (Daubeny, 1966). Genes *Ag₂* and *Ag₃* are dominant genes functioning in tandem to confer resistance and are derived from wild *Rubus strigosus* Michx. populations in eastern Canada (Daubeny and Stary, 1982). Sources of partial resistance to *A. agathonica*, as evidenced by the ability of aphids to colonize plants in small numbers, have also been identified (Daubeny, 1972; Kennedy et al., 1973), but the levels of resistance are less than that conferred by *Ag₁*, and so have received little attention. Until the 1990s, there was evidence for only a single biotype of *A. agathonica* (Converse et al., 1971). However, Daubeny and Anderson (1993) described the discovery of an *Ag₁* resistance-breaking strain in British Columbia, and new sources of resistance to *A. agathonica* are needed, not only to slow the spread of resistant biotypes, but also to help maintain the durability of existing resistance as well.

While black raspberry has been an important source of resistance *A. idaei* in Europe (Keep and Knight, 1967), strong resistance to *A. agathonica* has not been identified in

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R. occidentalis. Efforts to transfer resistance from red raspberry have been slow and have not resulted in any cultivars. The purpose of this study was to identify sources of strong resistance to *A. agathonica* in wild populations of black raspberry and to determine its inheritance.

Materials and Methods

PLANT MATERIALS AND APHIDS. During Summer 2006, friends and colleagues living in eastern North America, within the native distribution of *R. occidentalis*, were solicited to send seed or fruit from wild plants in their area. Additional seed was obtained in 2007 through a similar request and collecting trips across the southern and western edges of the native range (Hall et al., 2009). Through these efforts, seeds were obtained from more than 150 locations across the range, including 27 states and two Canadian provinces. From some locations, seed from multiple maternal clones were sent as separate samples and were kept separate from each other after arrival. From other locations, the seeds represented bulk fruit samples of many individuals (Table 1). Upon arrival, seeds were extracted from the fruit, dried, and stored in a cool dry place until scarification. Seed was also obtained from eight populations held at the National Clonal Germplasm Repository in Corvallis, OR. Seeds were scarified in concentrated sulfuric acid for 45 min in an ice bath, rinsed with sodium bicarbonate solution (Church and Dwight, Princeton, NJ), soaked for 6 d in a 3 g·L⁻¹ calcium hypochlorite solution (Sigma-Aldrich, St. Louis) with an excess of calcium hydroxide (Mallinckrodt, Phillipsburg, NJ), and then planted on moist peat and held at 4 °C for 8 weeks. Seedling trays were then placed on the mist bench with bottom heat at 24 °C for germination. After the emergence of their first true leaf, seedlings were planted in 72-cell trays (cell dimensions 4 × 4 × 6 cm), and then placed in the greenhouse under a 16-h daylength. When seedlings were ≈10 to 12 cm tall, they were repotted individually and then subjected to screening for aphid resistance in the greenhouse under similar conditions to those described above. Seedlings were watered regularly and given a liquid fertilizer (20N–8.7P–16.6K; Scotts, Marysville, OH) once per week throughout this period to promote fast growth and tender tissue attractive to aphids.

Alate and apterous *A. agathonica* were collected from a commercial red raspberry field and were raised in screened cages on the susceptible ‘Meeker’ red raspberry. Adult aphids from this colony were placed in petri dishes with a detached leaf and moist filter paper to isolate nymphs as they were born. Nymphs were removed from the petri dishes at 12-h intervals for 5 d and were transferred to virus-free plants of the susceptible black raspberry ‘Munger’ in a separate cage to ensure that aphids used for screening seedlings did not carry viruses from the field. This colony was maintained in a screened cage in the greenhouse under a 16-h daylength for the duration of the project. All aphids used in resistance screening came from this colony.

APHID RESISTANCE SCREENING. Between June and Aug. 2007, 5415 seedlings were screened for resistance to *A. agathonica* in the greenhouse. An additional 1140 wild seedlings were screened for resistance between Feb. and Apr. 2008. We began screening 72 seedlings from each seed lot for resistance, but quickly determined that seed lots could be adequately sampled for resistance with only 36 seedlings. Thus, in 27 seed lots, 72 seedlings were screened for resistance to *A. agathonica*, and in 151 seed lots, up to 36 seedlings were screened. Some seed lots experienced poor germination; in cases where fewer than 36 seedlings

were available, all seedlings were screened for resistance. In those populations where seed from different maternal clones at a location were sent as separate samples, 36 seedlings from each were screened for resistance for a total of up to 216 seedlings from some locations. The methods for screening plants for resistance to *A. agathonica* were similar to those previously used by others screening for resistance to aphids in red raspberry (Daubeney and Sary, 1982; Knight et al., 1959). Three apterous adult aphids were placed on the youngest leaves of each seedling and were allowed to remain undisturbed for 1 week. After 1 week, each seedling was examined for evidence of colonization. Plants with more than 10 aphids on them were scored as susceptible and were placed back on the bench. Plants with fewer than 10 aphids were marked and the total number of adult and nymph aphids was recorded before plants were placed back on the bench. Plants with fewer than three aphids had the appropriate number of adult aphids added to them to bring the number up to three. Four days after this initial inspection, this procedure was repeated. Plants assumed to be susceptible during the first inspection were re-examined to confirm susceptibility, while the procedure for plants with fewer than 10 aphids remained the same. This was repeated at 4-d intervals three more times for a total of five challenges. In this manner, plants that continually exhibited a non-preference reaction by supporting fewer than 10 aphids were identified under conditions of extreme aphid feeding pressure.

In Apr. 2008, a colony of *Amphorophora rubitoxica* Knowlton was started from aphids collected in the field, and was maintained on ‘Munger’ using the same procedures as outlined above. Single potted plants of ‘Munger’, ORUS 3778–1, ORUS 3817–1, and ORUS 4109–1 were simultaneously placed in the colony and were monitored over a 2-week period for colonization. This was repeated three times over the course of the summer to determine whether resistance to *A. agathonica* coincided with resistance to *A. rubitoxica*. Seedlings from the cross ORUS 4158–2 × ORUS 3021–2 were also screened for resistance to *A. rubitoxica* using the same procedures described above for *A. agathonica*.

F₁ POPULATIONS. Two aphid-resistant seedlings from ORUS 3778 (Simcoe, ON, Canada) and ORUS 3817 (Gardiner, ME) were selected for crossing the following year: ORUS 3778–1, ORUS 3778–2, ORUS 3817–1, and ORUS 3817–2 (a four-digit ORUS number followed by a dash and another number indicates a selection from within that cross). These plants were moved to large pots and were grown in the greenhouse until September, when they were moved outside. In Jan. 2008, these four plants were brought back into the greenhouse, along with potted plants of the susceptible cultivars Munger and Black Hawk. These plants were allowed to break dormancy and flower, at which time crosses between the resistant and susceptible genotypes were performed (Table 2). Fruit and seeds from these crosses were treated as described above and, after 2 weeks drying, were subjected to the same germination procedures. A subset of seedlings from each cross was screened in the greenhouse for resistance to *A. agathonica* using the procedures described above (Table 2). This procedure was repeated in Jan. 2009 with the single resistant seedling from Bath, MI (ORUS 4109–1), and an aphid susceptible selection, ORUS 3021–1.

BC₁ POPULATIONS. Five seedlings from each of four F₁ populations ORUS 4153, ORUS 4155, ORUS 4157, and ORUS 4158 were moved to large pots in Sept. 2008. The plants were fertilized weekly with a liquid fertilizer (20N–8.7P–16.6K; Scott’s, Marysville, OH) and kept in a warm greenhouse under a 16-h daylength until 21 Nov. 2008 to promote growth and

Table 1. USDA-ARS PI number, breeding program cross identification (ORUS) number, general provenance, and results from screening 132 wild black raspberry populations for resistance to the North American large raspberry aphid.

PI no.	ORUS no.	Provenance	Susceptible (no.)	Resistant (no.)
653296	4123	Mentone, AL	36	0
Not in GRIN ^z	4184	Eureka Springs, AR	72	0
653327	3779	Litchfield County, CT	72	0
652975	4117	Clayton, GA	36	0
652976	4119	Clayton, GA	36	0
653294	4120	Clayton, GA	36	0
653298	4122	Dahlongega, GA	36	0
652977	4121	Union County, GA	36	0
653328	3780	Story County, IA	72	0
Not in GRIN	3789	Arenzeville, IL	6	0
653329	3781	Iroquois County, IL	70	0
553949	3946	Waukegan County, IL	4	0
653331	3796	Greene County, IN	72	0
653335	3800	Greene County, IN	36	0
653332	3797	Hendricks County, IN	36	0
653330	3794	Putnam County, IN	36	0
653333	3798	Sullivan County, IN	72	0
653334	3799	Vigo County, IN	36	0
653336	3801	southern Indiana	72	0
652984	4126	Alma, KS	6	0
653299	4124	Bonner Springs, KS	27	0
653303	4129	Fort Riley, KS	10	0
653301	4127	Manhattan, KS	2	0
651846	4130	Minneapolis, KS	16	0
653302	4128	Ogden, KS	3	0
653300	4125	Perry Lake, KS	3	0
651848	3802	Fayette County, KY	72	0
653337	3803	Berkshire County, MA	72	0
653338	3804	Berkshire County, MA	36	0
653343	3811	Allegany County, MD	36	0
653344	3812	Anne Arundel County, MD	36	0
Not in GRIN	3809	Dorchester County, MD	4	0
653341	3808	Harford County, MD	72	0
653342	3810	Howard County, MD	36	0
Not in GRIN	3806	Howard County, MD	36	0
653339	3805	Washington County, MD	36	0
653340	3807	Washington County, MD	72	0
653350	3821	Camden, ME	72	0
653349	3820	East Vassalboro, ME	72	0
653347	3817	Gardiner, ME	0	72
653348	3819	Hallowell, ME	36	0
651849	3815	Monmouth, ME	36	0
653345	3814	Orono, ME	36	0
653346	3816	West Kennebunk, ME	72	0
Not in GRIN	4109	Bath, MI	35	1
Not in GRIN	4110	Benton Harbor, MI	36	0
553765	3948	Fred Russ State Forest, MI	14	0
553766	3949	Fred Russ State Forest, MI	8	0
Not in GRIN	4111	Grand Ledge, MI	36	0
553764	3947	Oak Grove, MI	13	0
Not in GRIN	4112	Okemos, MI	36	0
653323	4149	Belgrade, MN	15	0
653321	4148	Big Stone Lake National Wildlife Refuge, MN	29	0
651847	4147	Big Stone Lake State Park, MN	36	0
653351	3823	Cass County, MN	36	0
651851	3827	Dakota County, MN	72	0

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Table 1. Continued.

PI no.	ORUS no.	Provenance	Susceptible (no.)	Resistant (no.)
653354	3828	Dakota County, MN	71	0
653355	3829	Dakota County, MN	36	0
653324	4150	Hasty, MN	11	0
651850	3824	Ramsey County, MN	36	0
653353	3826	Ramsey County, MN	72	0
Not in GRIN	3833	Cassville, MO	72	0
651852	3830	Fordland, MO	72	0
653356	3832	Fordland, MO	35	0
653357	3835	Madison County, NC	36	0
653358	3837	Rutherford County, NC	36	0
653359	3838	Rutherford County, NC	34	0
653311	4139	Chadron, NE	4	0
653310	4138	Chadron State Park, NE	36	0
653308	4136	Halsey, NE	36	0
653305	4133	North Loup State Recreation Area, NE	19	0
606476	3952	Otoe County, NE	3	0
653306	4134	Pibel Lake State Recreation Area, NE	4	0
653309	4137	Valentine, NE	36	0
653307	4135	Victoria Springs State Recreation Area, NE	18	0
638243	3955	Manasquan Reservoir, NJ	10	0
638244	3956	Tom's River, NJ	12	0
653363	3843	Columbia County, NY	35	0
653362	3842	Dutchess County, NY	34	0
653360	3839	Ontario County, NY	72	0
653361	3840	Ontario County, NY	35	0
Not in GRIN	3841	Ontario County, NY	35	0
618560	3951	Poughkeepsie, NY	10	0
653364–653367 ^y	3844–3848 ^x	Yates County, NY	176	0
653368	3849	Clermont County, OH	36	0
Not in GRIN	4107	Hilliard, OH	36	0
Not in GRIN	4108	Newton Falls, OH	24	0
653372	3854–3855	Center County, PA	72	0
653373	3856	Center County, PA	36	0
653369	3851	Chester County, PA	72	0
653370	3852	Greene County, PA	29	0
653371	3853	Somerset County, PA	36	0
Not in GRIN	4185	Charlestown, RI	5	0
652971	4113	Glassy Mountain, SC	36	0
652973	4114	Glassy Mountain, SC	36	0
652974, 653292	4115–4116	Rich Mountain, SC	72	0
653315	4142	Clay County State Park, SD	14	0
653318	4145	East Sioux Falls, SD	2	1
653317	4144	Newton Hills State Park, SD	36	0
653319	4146	Oakwood Lakes State Park, SD	8	0
652988	4140	Pease Creek State Recreation Area, SD	11	0
653316	4143	Union Grove State Park, SD	13	0
653314	4141	Yankton, SD	29	0
653389	3904–3905	Cannon County, TN	72	0
653395	3914–3915	Cheatham County, TN	72	0
653374	3857–3858	Davidson County, TN	72	0
653375	3859–3863	Davidson County, TN	180	0
653376	3864–3865	Davidson County, TN	72	0
653377	3866–3867	Dekalb County, TN	108	0
653378	3868–3870	Dekalb County, TN	108	0
653379	3871	Dekalb County, TN	72	0
653380	3872–3876	Dekalb County, TN	175	0

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Table 1. Continued.

PI no.	ORUS no.	Provenance	Susceptible (no.)	Resistant (no.)
653381	3877–3882	Dekalb County, TN	216	0
653384	3888–3891	Grundy County, TN	144	0
653385	3892–3895	Grundy County, TN	140	0
653398	3919	Henderson County, TN	36	0
653396	3916	Unicoi County, TN	36	0
653397	3918	Unicoi County, TN	36	0
653382	3883	Van Buren County, TN	36	0
653390	3906–3907	Van Buren County, TN	72	0
653392	3909–3910	Van Buren County, TN	72	0
653393	3911	Van Buren County, TN	36	0
653394	3912–3913	Van Buren County, TN	72	0
653383	3884–3887	Warren County, TN	144	0
653386	3896–3899	Warren County, TN	144	0
653387–653388	3900–3903	Warren County, TN	108	0
653399	3926–3928	Columbia County, WI	108	0
653401	3930	Inwood, WV	72	0
653402	3931	Preston County, WV	36	0
653400	3929	Shepherdstown, WV	72	0
653325	3777	Mactaquac, NB, Canada	72	0
653326	3778	Simcoe, ON, Canada	38	34

^zAccessions not yet available through the USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network (GRIN).

^yMultiple PI numbers indicate separate seed lots from this location in GRIN.

^xMultiple ORUS numbers indicate seed lots from multiple maternal clones at this location were kept and evaluated separately.

Table 2. Summary of crosses in 2008 and 2009 involving black raspberry selections resistant to the North American large raspberry aphid, showing breeding program cross identification (ORUS) numbers and segregation for resistance.

Yr	ORUS no.	Parents	Postulated genotypes of parents	Resistant (no.)	Susceptible (no.)	Expected ratio	Chi-square	P
2008	ORUS 4153	'Black Hawk' × ORUS 3778–1 ^z	<i>ag₄ag₄ × Ag₄Ag₄</i>	36	0	1:0		
2008	ORUS 4154	ORUS 3778–2 × 'Black Hawk'	<i>Ag₄Ag₄ × ag₄ag₄</i>	18	0	1:0		
2008	ORUS 4155	'Munger' × ORUS 3778–2	<i>ag₄ag₄ × Ag₄Ag₄</i>	72	0	1:0		
2008	ORUS 4156	'Munger' × ORUS 3817–1	<i>ag₅ag₅ × Ag₅Ag₅</i>	36	0	1:0		
2008	ORUS 4157	ORUS 3817–1 × 'Black Hawk'	<i>Ag₅Ag₅ × ag₅ag₅</i>	54	0	1:0		
2008	ORUS 4158	ORUS 3817–2 × 'Black Hawk'	<i>Ag₅Ag₅ × ag₅ag₅</i>	18	0	1:0		
2008	ORUS 4159	'Black Hawk' × ORUS 3817–2	<i>ag₅ag₅ × Ag₅Ag₅</i>	18	0	1:0		
2009	ORUS 4304	ORUS 4158–2 × ORUS 3021–2	<i>Ag₅ag₅ × ag₅ag₅</i>	65	37	1:1	7.7	0.006
2009	ORUS 4305	ORUS 3021–2 × ORUS 4153–1	<i>ag₄ag₄ × Ag₄ag₄</i>	36	36	1:1	0.0	1.0
2009	ORUS 4306	'Jewel' × ORUS 4153–1	<i>ag₄ag₄ × Ag₄ag₄</i>	14	14	1:1	0.0	1.0
2009	ORUS 4310	ORUS 3021–1 × ORUS 4109–1	<i>rr × Rr</i>	36	36	1:1	0.0	1.0

^zA four-digit ORUS number followed by a dash and another number indicates a selection from within that cross.

maturity. These seedlings were then treated to induce flower bud initiation and dormancy before being brought back into the greenhouse on 1 Apr. 2009 to begin flowering. In early May, crosses were performed between these plants and field-grown plants of ORUS 3021–2 and 'Jewel' (Table 2). Seeds and seedlings from these crosses were treated in the same manner as above and in Dec. 2009, seedlings were screened for aphid resistance as already described.

STATISTICS. Chi-square tests to determine goodness-of-fit and probability of observed segregation ratios were performed using SAS (version 9.1; SAS Institute, Cary, NC).

Results and Discussion

Greenhouse inoculation of black raspberry seedlings with *A. agathonica* and *A. rubitoxica* resulted in colonization of susceptible plants. Susceptible plants typically supported 20 to

30 aphids after the first week, and a few hundred after 3 weeks. This was observed throughout the course of the screening periods and allowed resistant genotypes to be readily identified.

Table 1 lists the populations screened, their origin, and the number of seedlings found to have strong resistance to *A. agathonica*. Of the 132 populations screened, strong resistance was confirmed in only three: ORUS 3778, ORUS 3817, and ORUS 4109. In addition, a single seedling from South Dakota, ORUS 4145–1, was tentatively identified as resistant. Due to difficulties propagating this selection, its resistance has not yet been confirmed, and crosses involving ORUS 4145–1 to date have been unsuccessful. Intermediate levels of colonization were noted on one or more plants from several other locations, including ORUS 3827, ORUS 3844, ORUS 3848, ORUS 3854, ORUS 3855, and ORUS 4124. These may represent sources of quantitative resistance, simply inherited weaker resistance, or may be an artifact of changing environmental conditions and

aphid populations in the greenhouse during the 3 months in which screening took place. The 34 resistant seedlings of the 72 tested from ORUS 3778 averaged 1.9 aphids per plant after 1 week and 3.5 aphids per plant per inspection over the course of the 23-d screening period (data not shown). All 72 of the seedlings of ORUS 3817 were resistant. After 1 week, these plants averaged 0.9 aphids per plant and averaged 2.1 aphids per plant per inspection (data not shown) over the screening period. The single resistant seedling identified from ORUS 4109 supported no aphids on each of the first four examinations, and was found to have only two aphids on the final examination.

To confirm their resistance, three selections (ORUS 4109-1, ORUS 3778-1, and ORUS 3817-1) and 'Munger' (as a susceptible control) were propagated and five plants of each were screened for aphid resistance in a completely randomized trial. As expected, 'Munger' was the only plant to be colonized in this trial. Monitoring of resistant seedlings of ORUS 3778 and ORUS 3817 in the field in Corvallis, OR, during Spring and Summer 2008 and 2009 failed to detect *A. agathonica*. While *A. agathonica* numbers are fairly low in Corvallis, and vary greatly from year to year, they were found on susceptible black raspberry seedlings in some adjacent plots in 2009, indicating their presence in the field.

Seedlings from the crosses of ORUS 3778-1, ORUS 3778-2, ORUS 3817-1, and ORUS 3817-2 with 'Munger' and 'Black Hawk' were all resistant to colonization by *A. agathonica* when screened in the greenhouse (Table 2). This is consistent with the hypothesis that resistance from these two sources is controlled by dominant alleles and that all four of these resistant selections are homozygous. Crossing these resistant progeny back to susceptible elite germplasm resulted in segregation for resistance to *A. agathonica*. Both crosses involving resistance originating from ORUS 3778 segregated 1:1 for aphid resistance in the BC₁ generation (Table 2), confirming that resistance from this source is controlled by a dominant allele at a single locus. The cross ORUS 3021-1 × ORUS 4109-1 also segregated 1:1 (Table 2), confirming that resistance in ORUS 4109-1 is also controlled by a single dominant gene for which ORUS 4109-1 is heterozygous. Due to a lack of flowers on juvenile plants, only a single BC₁ cross involving resistance originating from ORUS 3817 was possible. Progeny of the cross ORUS 4158-2 × ORUS 3021-2 did not segregate 1:1 as expected. Instead, an excess of resistant plants was observed. Nearly 64% of the progeny from this cross were resistant to colonization by *A. agathonica*. Two possible explanations for this observation are linkage to a lethal recessive allele originating in susceptible grandparents or a gene duplication event creating two loci acting independently to confer resistance in ORUS 4158-2. Susceptibility to colonization by *A. agathonica* in this cross comes from 'Black Hawk' and 'Jewel' as grandparents on opposite sides. If susceptibility were linked to a lethal recessive allele shared by 'Black Hawk' and 'Jewel', this would cause an excess of resistant plants as observed. This scenario seems plausible given that black raspberry cultivars appear to be closely related (Dossett et al., 2010), and that these two cultivars also share a rare recessive allele for non-glaucous canes (Dossett, 2007). Alternatively, duplication of this locus could also cause an excess of resistant progeny similar to that observed by Lunde et al. (2006) in 'Zimmerman' hazelnut and resistance to eastern filbert blight. If the duplicated loci are unlinked, then 3:1 segregation for resistance should be observed. Linkage of the duplicated loci would result in some lesser percentage of resistant progeny,

depending on the tightness of the linkage. Further study is needed to determine the exact reason for excess resistant progeny in this cross. Observation of segregation ratios in progeny of siblings of ORUS 4158-2 as well as in crosses involving resistant progeny of ORUS 4158-2 may clarify the cause of this result; however, it is clear from the data presented that resistance to *A. agathonica* is dominant to susceptibility.

When single plants of the *A. agathonica*-resistant ORUS 3778-1 and ORUS 3817-1 and the susceptible 'Munger' were caged in a colony of *A. rubitoxica*, 'Munger' and ORUS 3778-1 became readily colonized while ORUS 3817-1 was avoided entirely (data not shown). These results were echoed by observations from an inadvertent infestation of this aphid species in the greenhouse in late September and Oct. 2008. Seedlings of ORUS 3778-1 and ORUS 3778-2 crossed with 'Black Hawk' and 'Munger' became colonized by these aphids and the characteristic chlorotic lesions from their feeding (Stace-Smith, 1954) were present on the leaves. Seedlings of ORUS 3817-1 and ORUS 3817-2 crossed with 'Black Hawk' and 'Munger' remained free of aphids, and chlorotic lesions failed to appear. Testing in Dec. 2009 showed that resistance to *A. rubitoxica* and *A. agathonica* cosegregated in the cross ORUS 4158-2 × ORUS 3021-2. *Amphorophora rubitoxica* is not a known virus vector and consequently is of little concern to growers in the Pacific northwestern U.S. However, these results strongly suggest that resistance to *A. agathonica* in ORUS 3778 and ORUS 3817 is controlled by separate genes (designated *Ag₄* and *Ag₅*, respectively), with *Ag₅* from ORUS 3817 also conferring resistance to *A. rubitoxica*. Resistance to *A. agathonica* in ORUS 4109-1 is not effective against *A. rubitoxica* and cannot be distinguished from *Ag₄*. A lack of resistance found in the populations collected near this location suggests that resistance in ORUS 4109-1 may be conferred by a unique gene, and further investigation is necessary to determine if this is the case.

The occurrence of resistance to *A. agathonica* in *R. occidentalis* is of great value to black raspberry breeders looking for resistance to BRNV and other aphid vectored viruses in North America. While the data suggest the presence of minor gene aphid resistance in some wild black raspberry populations, further study is needed to determine its inheritance and the efficacy of using these sources successfully. Because it only takes a very small number of aphids to colonize plants and spread these viruses, partial resistance may have to be combined from many populations to achieve the desired level of control, and even then may not be sufficient. Work in Scotland has shown that even partial aphid resistance slows the spread of aphid transmitted viruses in red raspberry field plots (Jones, 1976, 1979). However, Stace-Smith (1960) showed that partial resistance was not enough to reduce virus transmission rates in greenhouse experiments with red raspberry.

The use of *Ag₄* and *Ag₅* together may increase their durability in new cultivars, reducing the likelihood of new resistance-breaking aphid biotypes from appearing, particularly if used in conjunction with minor gene resistance sources. This is an important consideration. Birch et al. (2005) indicate that every type of major gene aphid resistance in the United Kingdom has been broken while minor gene resistance has remained durable, though less effective. *Ag₄* and *Ag₅* are also of potential value to red raspberry breeders as they offer two new sources of resistance that may be incorporated into breeding lines in much the same way as *A₁₀* (Keep and Knight, 1967). Screening wild black raspberry populations for additional sources of aphid

resistance is worthwhile. These data suggest that the range has not been exhaustively sampled for diversity. Despite the resistance of all 72 plants sampled from Gardiner, ME, aphid resistance was not detected in three other nearby locations. To date, every source of strong aphid resistance identified in *R. occidentalis*, and nearly all those with some partial resistance, originate in the northern half of the species' range where raspberry aphids are typically more abundant and indicate this area may be a good target for further exploration.

Small quantities of seed from most of the populations studied are available from the USDA-ARS National Clonal Germplasm Repository in Corvallis, OR. Plants of ORUS 3778-1 (PI 658505), ORUS 3817-1 (PI 658506), and ORUS 4109-1 (PI 659143) will also be available by request.

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