Invasive Plant Science and Management 2017 10:143–154

Invasive Blackberry Species in Oregon: Their Identity and Susceptibility to Rust Disease and the Implications for Biological Control

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Two of the five species of European blackberry (*Rubus fruticosus* L. aggregate) along the West Coast of the United States are considered invasive. They are also similar in appearance. Biological control of invasive blackberry by *Phragmidium violaceum*, causal agent of a rust disease, had been under consideration when rust-diseased blackberry was discovered in Oregon in 2005. An investigation was initiated to determine whether this disease would be an important factor affecting population density of these blackberries. Surveys were made over a 5-yr period at more than 30 field sites in the Willamette Valley and along the Pacific coast of Oregon. Diseased and nondiseased blackberry specimens were collected for artificial greenhouse inoculations and for identification. The two blackberry species, *Rubus armeniacus* and *R. praecox*, were identified as the most invasive. They were readily distinguished morphologically on the basis of inflorescence and flower characteristics and to a certain extent by differences in primocane leaf and leaflet shape. Artificial greenhouse inoculation studies revealed that *R. praecox* was susceptible to the rust disease and that *R. armeniacus* was not. These results were confirmed during a field survey. Results of this investigation revealed that the rust disease will not be effective for biological control of *R. armeniacus* and other approaches to management of this particular species will be required.

Nomenclature: European blackberry, *R. fruticosus* L. aggregate, RUBFR; Armenian (=Himalaya) blackberry, *Rubus armeniacus* Focke, RUBDI; *R. praecox* Bertol. sensu lato; *R. anglocandicans* A. Newton; *Phragmidium violaceum* (Schultz) G. Winter.

Key words: Biological control, cutleaf blackberry (*R. laciniatus* Willd.), rust disease, Uredinales, Rosaceae.

Until recently, only three species of *Rubus* were recognized as naturalized along the West Coast of the United States. These are: (1) *R. armeniacus* Focke (= *R. discolor* auct. non Weihe & Nees and *R. procerus* auct. non P. J. Müll. ex Boulay), commonly known as Armenian, Himalaya, or Himalayan blackberry; (2) *R. laciniatus* Willd., commonly known as cutleaf blackberry; and (3) *R. ulmifolius* Schott (= *R. discolor* Weihe & Nees), commonly known as elmleaf blackberry (Alice 2015; DiTomaso and Healy 2007). Using primarily molecular characteristics, Clark et al. (2013) substantiated the presence of these species and documented the establishment of two others: *R. vestitus* Weihe, also described earlier from Salem, OR, by Bailey (1945); and a cryptic, but genetically distinct species from northern California, Oregon, and Washington, which they identified as *R. anglocandicans* A. Newton. Because the putative *R. anglocandicans* is similar in appearance to and is frequently sympatric with *R. armeniacus*, it is also generally known as Armenian blackberry. According to Clark et al. (2013), the most common naturalized species along the Pacific coast of the United States is *R. armeniacus*, ranging in distribution from California to Washington State. The greatest diversity among these species was at a site near Eugene, OR (Clark et al. 2013).

DOI: 10.1017/inp.2017.12

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Management Implications

Identifying invasive blackberry species along the Pacific coast of North America makes it possible to determine the importance of each species in the invasion process. Clarifying morphological differences facilitates field identification, thus making it possible to now map distributions of each, to determine the relative importance of each, to measure shifts in population densities over time, and, although *Rubus armeniacus* and *R. praecox* are sympatric, to clarify subtleties in habitat and environmental preferences that may affect strategies for management. Determination that the two species differ in susceptibility to the rust disease caused by *Phragmidium violaceum* affects implementation of biological control measures. The fact that the rust disease is not effective on *R. armeniacus* and requires application of other management strategies. Also, development of biological control of this species will necessitate searches for agents other than *P. violoaceum* in its native range.

Discovery of *R. anglocandicans* was unexpected (Clark et al. 2013). The name *R. anglocandicans* was applied by Clark et al. (2013) to these newly recognized U.S. accessions because they clustered with Australian accessions reported under this specific epithet (Evans and Weber 2003). The name for Australian populations was assigned on the basis of banding patterns from an M13/*Hae*III DNA digest protocol that were identical to those of *R. anglocandicans* from the United Kingdom (Evans and Weber 2003). Although the U.S. and Australian accessions were found to be similar on the basis of molecular data, they did not cluster with accessions of *R. anglocandicans* from the United Kingdom in the study by Clark et al. (2013), leading to the conclusion that these two groups (Australian/U.S. vs. UK) were from different asexual lineages. Evans and colleagues (Evans and Weber 2003; Evans et al. 2007) described difficulty in assigning the name “anglocandicans” to their accessions. Despite this, the name *R. anglocandicans* was applied by Clark et al. (2013) to the U.S. material.

European blackberry is invasive in Chile, Australia, and the United States. For this reason, biological control using *Phragmidium violaceum* (Schultz) G. Winter, the cause of a rust disease, was pursued both in Chile (Oehrens and Gonzalez 1974; Rejmánek 2015) and in Australia. Two strains were introduced originally into Australia, one illegally in 1984 and a second after a complete risk assessment (Mahr and Bruzzese 1998). Variability in disease response was noted within Australian “European blackberry” after introduction of the two rust fungal strains (Evans et al. 2005). Further investigation revealed that “European blackberry” in Australia is a complex, including “at least 15 polyploid agamospecies… and one diploid, sexual species” (Evans et al. 1998, 2007). *Rubus anglocandicans* was considered to be the most widespread of these (Evans and Weber 2003). To improve management of European invasive blackberry in Australia, eight additional strains of the rust fungus were released in 2004 (Morin et al. 2006, 2011).

Based upon the Australian experience and considering the magnitude of the invasive blackberry problem along the Pacific coast of North America, *P. violaceum* has also been of interest for biological control in the United States (Bennett 2007; DiTomaso and Healy 2007; Peters 2012). A strain of *P. violaceum* was discovered in Oregon in 2005 (Osterbauer et al. 2005), before formal U.S. evaluations had been initiated. Anecdotally, observations from 2004 and surveys in 2005 revealed severe dieback and death of blackberry that was attributed to the rust disease (Osterbauer et al. 2005). There was also evidence of diseased *R. laciniatus* at that time, including in commercially important ‘Thornless Evergreen’ and ‘Everthornless’ cultivars of *R. laciniatus* (Johnson and Mahaffee 2010; Osterbauer et al. 2005). More recently, rust disease was described on both *R. armeniacus* and *R. laciniatus* in Canada (Callan et al. 2011).

As in Australia, both variability in disease severity and incidence were noted in the United States by Johnson and Mahaffee (2010), who speculated about a “minor biotype” of *R. armeniacus* that was highly susceptible to *P. violaceum* in Oregon. They identified the remainder, i.e., “the more predominant biotype,” as not susceptible. Objectives of this research were to evaluate the effects of *P. violaceum* from Oregon on invasive *Rubus* species and to consider its potential for biological control of invasive blackberry along the West Coast of the United States

Materials and Methods

Clarifying susceptibility of introduced *Rubus* species was accomplished in two research thrusts. The first involved field surveys, which led to the second phase, i.e., the collection of plant material for greenhouse inoculation and study. It became necessary to identify specimens morphologically in order to fully understand differences noted in disease response in both the field and the greenhouse.

Field Survey. Between 2005 and 2009, field observations were made periodically at blackberry sites selected for the most part on the basis of symptomatic plants but also including some identified by collectors as “resistant” or not diseased. Specimens from these sites were sent to the USDA, ARS, Foreign Disease–Weed Science Research Unit (FDWSRU) for processing. This resulted in accumulation of several living blackberry clones and isolates of the pathogen, including the establishment of the disease under greenhouse conditions. Greenhouse inoculations were initiated in 2009.

To more fully understand this disease in Oregon within populations of feral blackberry, a random sampling was made in 2010 that included visits to more than 30 sites

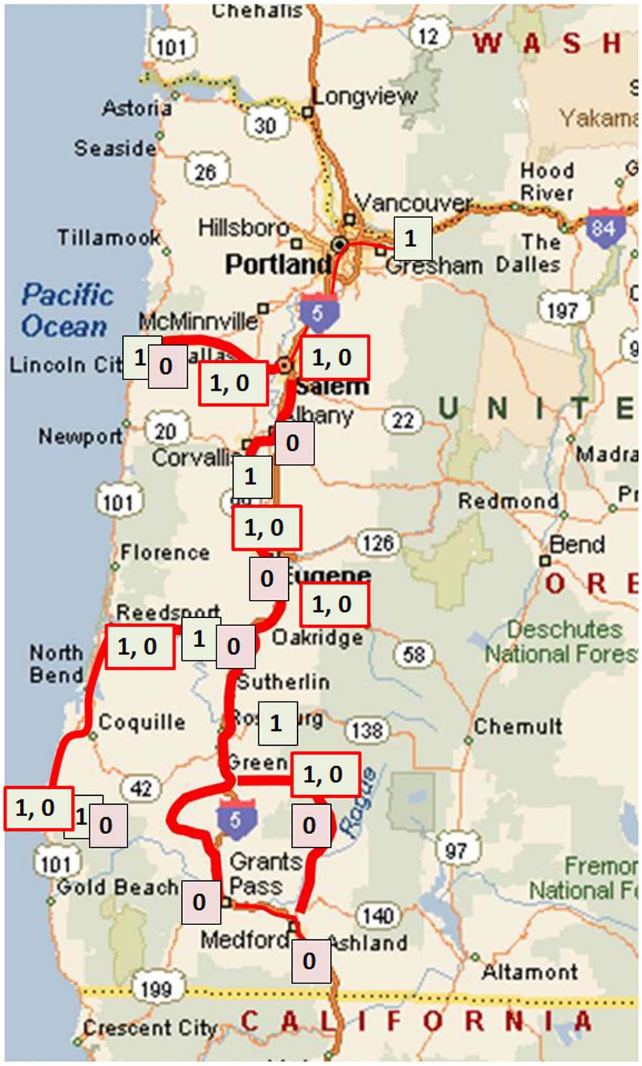

Figure 1. Survey routes for invasive blackberry in Oregon, 2009–2015, showing locations for plants that were not diseased ("0," *Rubus armeniacus*) or diseased by *Phragmidium violaceum* ("1," *R. praecox* s. lat.) in the field and/or in greenhouse tests. Not shown are locations for *R. laciniatus* or *R. vestitus*.

during the week of August 2. The area surveyed was west of the Cascade Mountains in Oregon, and ranged from Portland to Ashland (Figure 1), i.e., the Willamette Valley and sites along the Pacific coast of Oregon. The objective of site visits was to collect, if possible, one diseased and one "healthy" (i.e., nonsymptomatic) plant specimen at each location. Records for each site included GPS coordinates, text description of the location, notes on disease incidence, and photographs. Each plant specimen was trimmed at the field site, and the cut end was put into an Aquatube (Syndicate Sales, Kokomo, IN 46901) filled with water. Living specimens were then put into plastic bags and shipped overnight to the FDWSRU. Upon receipt, packages were opened and processed in the microbial-containment greenhouse and laboratory facility at the FDWSRU. Collection numbers were assigned to both plant and pathogen acquisitions. Pathogen samples were stored in a –80 C ultracold freezer. Plant samples were treated with a rooting hormone (Hormex® Liquid Concentrate plus Vitamin B1, Brooker Chemical Corporation via Maia Products, Chatsworth, CA 91313), put into rock wool (OASIS® Grower Solutions, Smithers-Oasis North America, Kent, OH 44240), and set into a misting tent for rooting. Canes of specimens rooted in containment were cut for removal following a protocol approved by regulators from the USDA, Animal and Plant Health Inspection Service (APHIS). It involved surface sterilization of cuttings (15% bleach for 5 min), removal from containment, and transfer to a conventional greenhouse for rooting and propagation. In addition to field samples from Oregon, one accession of *R. praecox* (Oehrens and Gonzalez 1974; Rejmánek 2015) was supplied by L. Ciampi (Univ. Austral de Chile, Valdivia), and two accessions each of *R. praecox* and *R. armeniacus* were supplied from the Czech Republic by M. Sochor and B. Trávníček.

Rooted specimens for the production of test canes were transplanted into 15-cm clay pots filled with a standard artificial soil mix of peat (41%), bark (11%), perlite (23%), vermiculite (23%), sand (2%), and Micromax® trace minerals (Scotts Miracle-Gro, Marysville, OH 43040). Herbarium material was not collected in 2010.

Field sites were revisited by colleagues from the Oregon Department of Agriculture in 2011 and 2012, who noted status of disease and provided additional pathogen acquisitions and herbarium specimens. In 2013, 2014, and 2015, collecting trips were made to sites visited originally in 2010 and selected on the basis of results from greenhouse inoculations. This enabled confirmation of earlier findings, the development of additional notes, photographic records, and the collection of additional plant and pathogen samples for tests (locations illustrated in Supplemental Figure 1). Plant material was also specifically collected for herbarium records (Supplemental Figure 2a–i) and for both morphological characterization and measurement, e.g., of leaves (Figure 2), which facilitated identification of species. In 2014 and 2015, petal samples were collected on cellophane tape that was put onto index cards (Supplemental Figure 3). Petals were measured for length and width.

Greenhouse Inoculations. The greenhouse investigation was initiated in 2009, when three isolates were established and increased after artificial inoculation. The isolate FDWSRU #09-020, from very heavily diseased blackberry along the Elk River (Figure 3), was used for most of the greenhouse studies. More recently, inoculations involved FDWSRU #09-010, collected at the Langlois Store site.

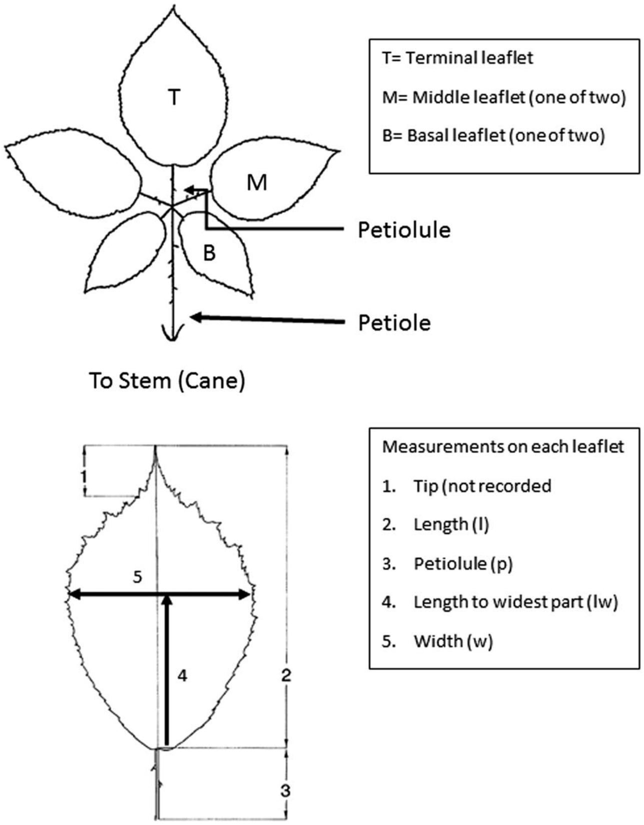

Figure 2. Drawings of leaf and leaflets, modified from Weber (1995), illustrating measurements taken from herbarium specimens.

Plant accessions were increased by periodic rooting, as described, and maintained in a conventional, locked greenhouse in 15-cm clay pots, according to APHIS permit. For disease-response studies, three to five canes from as many as five accessions were cut and placed in water in 1- or 2-L flasks. Each flask (a “bouquet”) contained a representative set of accessions (replication within inoculation), and there were three to five flasks per inoculation (Figure 4). Canes of a known susceptible accession from Lincoln City, OR, were included as a positive control in each bouquet. Attempts were made to inoculate each accession on at least three separate occasions (=3 repetitions).

Inoculum was used either fresh or after ultracold storage. A suspension of urediniospores in water was sprayed onto bouquets at the rate of 5 mg flask^{-1}, and inoculated canes were given two 16-h dew treatments at 18 C in the dark separated by an 8-h period at laboratory conditions outside of the dew chambers. Flasks were placed on a bench in a 25 C greenhouse, and canes were observed for symptoms. Data were recorded for pustules on the three most-diseased leaves and for both the total number of inoculated leaves and the number of symptomatic leaves. Incidence, i.e., proportion of diseased leaves, was calculated.

Figure 3. Heavily diseased leaf of invasive blackberry near the Elk River in Oregon. This isolate was used in the majority of greenhouse tests for susceptibility to disease.

Plant Identifications. Identifications were based on morphological characteristics as described by Edees and Newton (1998), Weber (1972, 1995), Zielinski (2004), and from the long-term taxonomical experience of the Czech coauthors with bramble populations in western and central Europe. Translations of German keys were provided by Christine Dieckhoff (USDA, ARS, Newark, DE). Identifications were based upon data collected from the field, from herbarium sheets, and from petal cards. Herbarium

Figure 4. Flasks of canes with test blackberry accessions (bouquets) for inoculation by *Phragmidium violaceum*.

sheets have been submitted to the L. H. Bailey Hortorium at Cornell University (BH), the Ada Hayden Herbarium at Iowa State University (ISU), the Oregon State University Herbarium (OSC), the University of California at Davis Herbarium, and the Palacký University herbarium (OL), Olomouc, Czech Republic.

Flow Cytometry. Ploidy level of invasive *Rubus* from Oregon was determined using flow cytometry. Accessions assessed were one specimen of *R. laciniatus* (072407-1), three specimens of *R. armeniacus* (080709-3, 080210-4, and 080310-1), and five specimens of *R. praecox* s. lat. (080410-1, 080410-3, 080510-9, 080510-12, and 071315-4). These accessions represent a range of locations in Oregon (Table 1), and those of *R. praecox* in particular represented a range of leaf morphological characteristics within these accessions.

Ploidy-level determinations were based on the relative fluorescence of stained nuclei using flow cytometry measurements of fresh leaves from a BD Accuri C6 (BD Biosciences, Franklin Lakes, NJ 07417) flow cytometer. As an internal standard, *Solanum lycopersicum* 'Stupické polní rané' (somatic cell DNA content $2C = 1.96$ pg; Doležel et al. 1989) was used. Leaf tissues of the sample and standard were chopped together with a razor blade in 0.5 ml LB01 buffer (Doležel et al., 1989; 15 mM Tris, 2 mM EDTA, 0.5 mM spermine tetrahydrochloride, 80 mM KCl, 20 mM NaCl, 0.1% Triton X-100, 30 g L^{-1} PVP40, and 550 µl L^{-1} 2-mercaptoethanol [$pH = 8.0$]). The suspension was filtered through a 42-µm nylon mesh and stained with 20 µl of propidium iodide. At least 3,000 particles were measured within the size limits of the sample and the standard only. BD Accuri C6 (BD Biosciences) software was used to calculate peak positions and coefficients of variation (CV). The highest CV value for accepted peaks was approximately 5.0%. For ploidy-level calibration, genotypes of *R. moschus* ($2n = 14$; chromosomes counted by Krahulcová and Holub 1997; documented by herbarium specimen OL no. r266/11 collected by Trávníček) and *R. bifrons* Vest ($2n = 28$; counted by Tesařová 2012; documented by OL specimen Dus2) were also measured.

Statistical Analyses. The mean incidence for each accession within a replication from greenhouse inoculations was used for data analysis. Results from at least two or, for the most part, three repetitions were included in the analysis of each accession. Incidence data were analyzed using Proc GLM (SAS Institute, Cary, NC 27513) to test the model: incidence = species. Data were tested for randomness and normality using Proc UNIVARIATE before analysis and transformed, if necessary. LSMeans were calculated from the GLM analysis and considered statistically significantly different if $P \leq 0.05$, based upon probability of differences (PDIFF) in SAS. In addition, the variable incidence was converted into a disease variable, with values of either "0" (= no disease) or "1" (= diseased) assigned to each accession within a replication. These data were analyzed using Proc GLIMMIX in SAS, modeled as a random variable from a beta distribution, and analyzed as a one-factor generalized linear model with species as the factor. Assumptions of the models were checked. Comparisons were done with Sidak-adjusted P-values so that the experiment-wise error was 0.05.

Results and Discussion

Plant Morphology, Identification, and Species Distribution. Four species of introduced *Rubus*, i.e., *R. armeniacus*, *R. laciniatus*, *R. vestitus*, and the cryptic species *R. praecox* s. lat., were found in Oregon during this survey. Two of these species, i.e., *R. laciniatus* and *R. vestitus*, were easily distinguished morphologically and were not particularly common. *Rubus laciniatus* was found at four of 33 sites, and *R. vestitus* was found at one (Table 1). Clark et al. (2013) reported that *R. ulmifolius* occurred only in California, and it was not found during the present survey in Oregon, possibly due to its preference for an oceanic climate (Kurtto et al. 2010; B Trávníček and M Sochor, personal observations).

The other two species were found to be very common, widely distributed, and invasive throughout Oregon west of the Cascade Mountains (Table 1; Figure 1). Both belong to the same tetraploid complex of *Rubus* series *Discolores*. Although *R. armeniacus* was described from central Europe as an invasive crop, it originated in the eastern Transcaucasian region (Focke 1910; Sochor 2016). *Rubus armeniacus* is probably a strictly asexual species with low intraspecific morphological variability. In contrast, *R. praecox* s. lat. is a European taxon that includes several very close, facultatively asexual lineages, which are usually grouped under the species name *R. praecox* (e.g., see Kurtto et al. 2010). Moreover, poor type material has led to many recent discussions about the proper application of this name (e.g., van de Beek 2014). Improved taxonomic understanding of this group is likely to develop in the near future.

Values of relative fluorescence of stained nuclei from the flow cytometry protocol of specimens from Oregon indicated tetraploid level of all *R. armeniacus* (three accessions; mean relative fluorescence ratio sample: standard $RF = 0.744 \pm 0.0016$ [SE]), all *R. praecox* s. lat. (five accessions; $RF = 0.765 \pm 0.0017$), and *R. laciniatus* (one accession; $RF = 0.738$). These results are in accordance with values reported for these species from Europe (Krahulcová et al. 2013; M Sochor, unpublished data). Unfortunately, no ploidy data have been reported for *R. anglocandicans* to date.

On the basis of morphological features that separate the two species, *R. armeniacus* was recorded in 24 (or 73%)

Table 1. Locations in Oregon (except where noted) that were surveyed and rust disease caused by *Phragmidium violaceum* observed both in the field and after artificial greenhouse inoculation.

			Field disease[a,b]				Greenhouse test[b,c]			
Location	Latitude	Longitude	A	P	L	V	A	P	L	V
Ashland	42.17429	−122.6697	–				–			
Creswell	43.91403	−123.0521	–				–			
Drain	43.64072	−123.2997	–				–			
Elk River, French Site	42.785	−124.481	–	+				+		
Elk River; Marsh Place	42.77114	−124.4611	–	+++			–	DNG		
Elk River; Fish Hatch	42.73823	−124.4029	–	+						
Fall Creek St. Rec. Area	43.95699	−122.7556	–	+	+					
Glide	43.30228	−123.119		+				+		
Glide Pond	43.309	−123.115		+				+		
I-5 Rest Area, Creswell	43.8529	−123.0208		+						
I-5 & Rt. 34	44.55613	−123.0643	–	+						
Langlois Market	42.92815	−124.4502	–	+		+	–			
Lincoln City, N; Rt. 101	44.99874	−123.9926	–	+						
Lincoln City, S; Rt. 101	44.92755	−12.40195		+						
Monroe Park	44.31296	−123.2956	–	+			–	+		
North Bend	43.41443	−124.2538	–	+						
Oak Knoll Golf Course	44.9336	−123.1631	–	+				+		
Oregon	Unknown location				+				+	
Placerville (CA)	38.68926	−120.7125	–							
Rt. 22, Perrydale Rd.	44.97129	−123.3044		+				+		
Rt. 38, Site 15	43.67256	−123.6910	–	+++				DNG		
Rt. 38, E of Reedsport	43.69431	−124.0699	–	+				+		
Rt. 99, N of Eugene	44.16428	−123.2015	–	+			–			
Rt. 99W & Payne Rd.	44.49006	−123.2718		+				+		
Rt. 138, S of Kellogg	43.46007	−123.4424		+				+		
Rt. 227, Apron	42.94456	−123.0134	–	+/–				+/+		
Rt. 227, Hedge	42.8739	−122.8885		+	–			+	+	
Rt. 227, Logging Rd.	42.78571	−122.8687	–	+	–		–			
Rt. 227, Pullout	42.97561	−123.1913	–	+						
Salem, Culver Rd.	44.90612	−122.9400	–	+						
Salem, Heritage Hill	44.89644	−122.9183	–	+						
Schroeder Park	42.43599	−123.3754	–				–			
Van Duzer State Park	45.03761	−123.8093	–	+			–			
Chile	Unknown location			+				+		
Staré Hutě, Czech Rep.	49.13722	17.29894		+				+		
Buchlovice, Czech Rep.	49.09278	17.32056		+				+		
Velehrad, Czech Rep.	49.11444	17.38722	–				–			
Olomouc, Czech Rep.	49.5775	17.28556	–				–			

[a] Field observations and identifications between 2009 and 2015.

[b] Abbreviations and symbols: A, *Rubus armeniacus*; P, *R. praecox* s. lat.; L, *R. laciniatus*; V, *R. vestitus*. Disease response: minus sign (–), no disease; plus sign (+), disease; triple plus sign (+++), heavily diseased; DNG, did not grow (accession lost). Two specimens were collected, one not symptomatic in the field (+/–). Both accessions were susceptible in greenhouse tests (+/+).

[c] Accessions that were collected, grown, and tested.

of the 33 locations, *R. praecox* was recorded in 27 (or 82%) of the locations, and of these, both species occurred at 19 (or 58%) of the locations (Table 1). These results suggest both species are nearly equal in occurrence, very common throughout the range of the survey, and frequently sympatric.

Table 2. Least-square means of petal measurements (mm) from *Rubus armeniacus*, *R. praecox* s. lat., and *R. vestitus* collected in Oregon.

	n			
Rubus sp.	Petals	Locations	Length[a]	Width[a]
armeniacus	23	8	14.5 a	12.3 a
praecox s. lat.	37	11	12.3 b	9.5 b
vestitus	4	1	13.5 ab	10.8 b

[a] Means followed by the same letter are not statistically different (P = 0.05).

Rubus armeniacus was distinguished by large pink (occasionally very pale pink, almost white) flowers that have large petals (Table 2; Figure 5), very long stamens (up to twice as long as styles), straight prickles on the inflorescence axis (Figure 6, left), and terminal primocane leaflets that are large and very broadly elliptical to nearly round (Figure 5), measuring 9.6 ± 0.5 cm long (mean ± confidence interval (c.i.), P = 0.05; Table 3). Inflorescences are usually large and loose (Figure 6, left). The terminal leaflet on primocanes often is cuspidate. Plants are large, often >2-m tall, robust, and with arching stems.

The second species, very similar in gross morphology to *R. armeniacus*, was identified as *R. praecox* s. lat., having medium-sized white flowers as suggested by petal size (Table 2), buds that are sometimes slightly pinkish, stamens only slightly longer than style, elliptical to broadly elliptical terminal leaflets that are smaller, 7.7 ± 0.5 cm long (c.i., P = 0.05; Table 3), and curved prickles on the inflorescence axis (Figures 6, right, 7). Inflorescences are moderate in size and more compact (Figure 6, right) than those of *R. armeniacus* (Figure 6, left).

Distinct morphological differences were noted between *R. praecox* and *R. armeniacus* in this study (Figures 5–8). Petals (Table 2) and terminal leaf sizes (Table 3) were significantly smaller for *R. praecox* compared with *R. armeniacus*. Even so, leaf size was not a particularly useful diagnostic in the field, considering overlap in the range of terminal leaflet dimensions (Table 3). There was tendency for all leaflets of *R. praecox* to be more narrowly elliptical than those of *R. armeniacus* (Table 3; Figure 8). Differences in prickle morphology in the inflorescences (or infructescences) and flower size and morphology, particularly relating to petal size and the length of stamens, were very useful in the field.

Figure 5. *Rubus armeniacus* from Monroe Park, OR, growing near *R. praecox* (illustrated in Figure 7): (A) single pink flower with very long stamens, (B) inflorescence axis with straight prickles and pink flowers showing long stamens, and (C) three leaves showing characteristic leaflet arrangement and morphology.

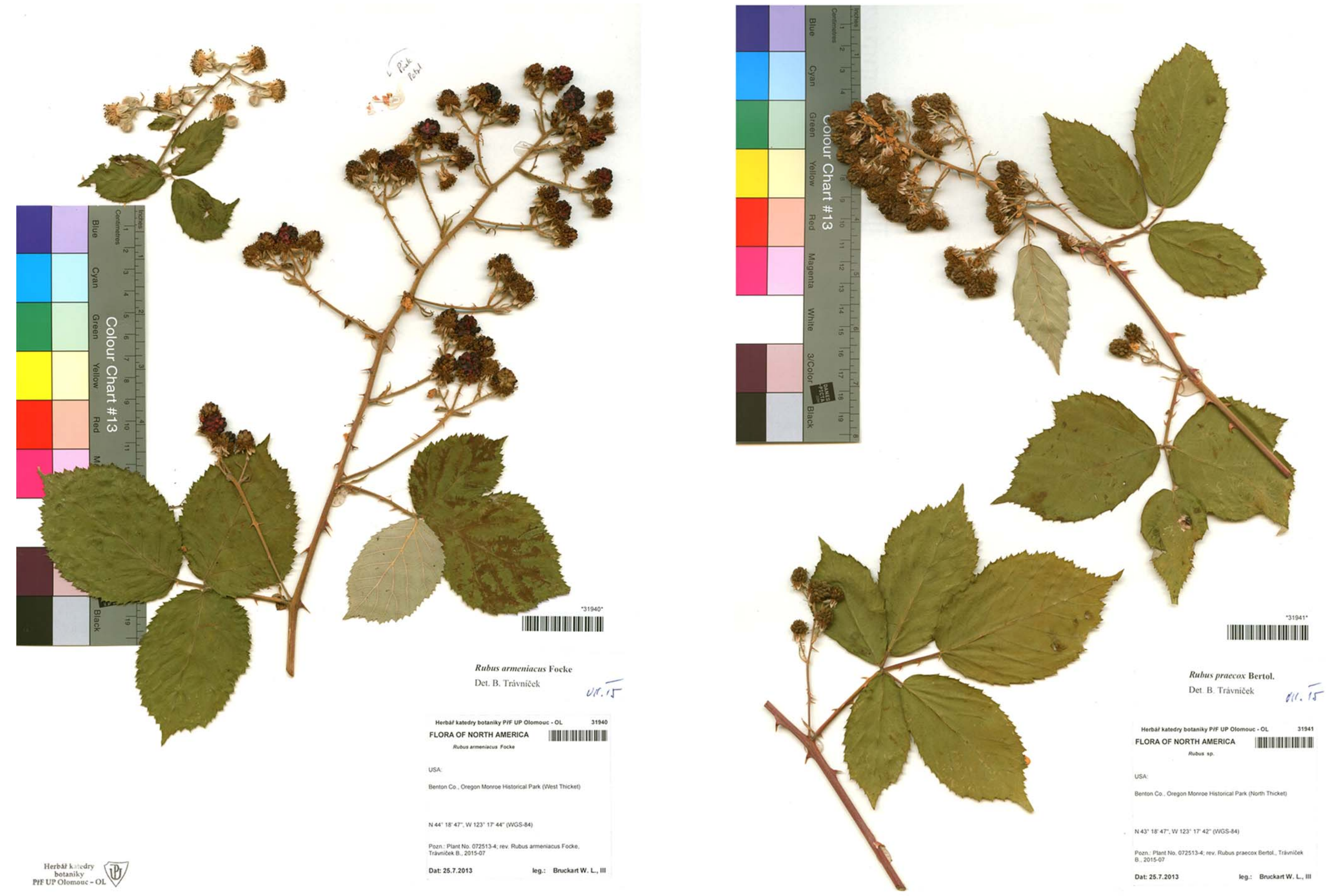

Figure 6. Herbarium sheets of inflorescences of *Rubus armeniacus* (left) and *R. praecox* s. lat., both from Monroe Park, OR.

Disease Responses. Results of the field survey and artificial greenhouse inoculations with *P. violaceum* revealed clear differences in susceptibility of invasive *Rubus* species in Oregon. The fact that *R. armeniacus*, of the two cryptic species, is not susceptible (Tables 1, 4; Figure 8) was unexpected. Similar differences were noted in tests with a Chilean accession of *R. praecox*, which was susceptible, and with four accessions from the Czech Republic, two of *R. armeniacus* that were not susceptible and two of *R. praecox* that were (Table 1). Similar results were reported from Germany by Schön (2014), who found that *R. bifrons*, *R. laciniatus*, and *R. praecox* were susceptible to rust disease caused by *P. violaceum*. It was also learned that *R. armeniacus* is not infected by *P. violaceum* in Germany (M Schön, personal communication).

Table 3. Least-square means (LSMs) and ranges of terminal leaflet measurements (cm) from *Rubus armeniacus*, *R. praecox* s. lat., and *R. vestitus* collected in Oregon.

	n		Length		Width	
Rubus sp.	Leaflets	Locations	LSM[a]	Range	LSM[a]	Range
armeniacus	26	7	9.6 b	7.5–12.5	7.4 b	5.9–9.8
praecox s. lat.	26	9	7.7 a	5.7–10.5	5.3 a	3.1–8.3
vestitus	6	1	8.0 b	8.1–13	8.0 b	5.2–9.9

[a] Means followed by the same letter are not statistically different (P = 0.05).

That *R. laciniatus* is susceptible was known from previous reports (Callan et al. 2011; Johnson and Mahaffee 2010; Osterbauer et al. 2005; Schön (2014), and such was confirmed in greenhouse tests (Table 4). *Rubus vestitus* was also found to be diseased in the field, but it was not tested in greenhouse studies (Tables 1, 4). The lack of disease as a diagnostic for *R. armeniacus* is useful, but it can be variable, including absent, on *R. praecox* from year to year. Thus, morphological features, rather than disease, should be used for field identifications.

Rubus anglocandicans. One issue is the correct name for the U.S. species identified by Clark et al. (2013) as "cryptic," and the presence of which was supported by results described in this paper. It has been identified as *R. praecox* s. lat. in this study, and it is likely the same species as that named *R. anglocandicans* by Clark et al. (2013), a name that was applied due to the fact that it clustered with Australian material named *R. anglocandicans* on the basis of M13/*Hae*III DNA digest banding patterns (Evans and Weber 2003). There are considerations that support *R. praecox* s. lat. as the correct name for these invasive bramble populations. In both Evans and Weber (2003) and Evans et al. (2007), accounts of uncertainty in reaching their determination of *R. anglocandicans* were presented. Clear differences noted between Australian and UK accessions of *R. anglocandicans* included lack of hairs on stems, terminal leaflets that are larger and more broadly elliptical (resembling that of *R. armeniacus*), and relatively longer petioles, among other features. Very clear difference was noted, particularly in petal size (Table 5), which was much larger than that of *R. anglocandicans* (Edees and Newton 1988).

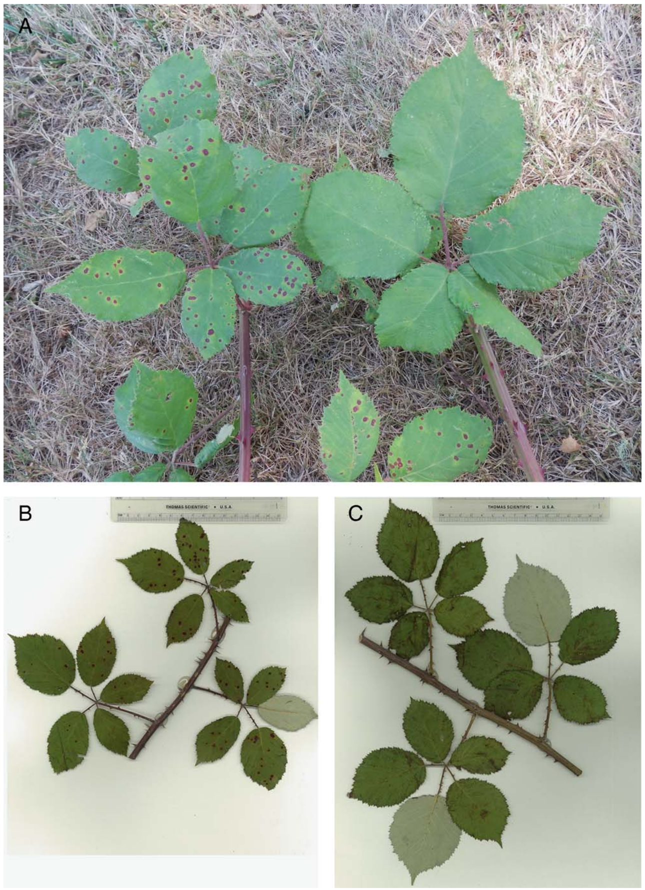

Figure 8. (A) Field specimens and (B, C) herbarium sheets of diseased *Rubus praecox* s. lat. (left) and *R. armeniacus* (right), collected within 20 m of each other.

Figure 7. *Rubus praecox* s. lat. from Monroe Park, OR, growing near *R. armeniacus* (illustrated in Figure 5): (A) two flowers with stamens of moderate length, (B) stem of inflorescence showing chacteristic curved prickles and flower and leaf morphology, and (C) a characteristic leaf with symptoms of *Phragmidium violaceum* rust disease.

Table 4. Means and mean comparisons of disease incidence (% infection) after greenhouse inoculations of *Rubus armeniacus*, *R. praecox* s. lat., and *R. laciniatus* with *Phragmidium violaceum* from Oregon.

Rubus sp.	Accessions	Infection[a] (%)
armeniacus	7	4.5 c
praecox s. lat.	10	64.3 a
laciniatus	2	32.0 b

[a] Means followed by the same letter are not statistically different at the 0.05 significance level.

Considering these factors, *R. praecox* s. lat. is the correct name for these blackberries.

Rubus armeniacus. Identity of *R. armeniacus* as invasive along the Pacific coast of the United States and Canada is strongly supported by reports in the literature. Ceska (1999) first applied this name on the basis of determinations by European expert batologists, who indicated that those epithets applied previously, i.e., *R. discolor* and *R. procerus*, were incorrect. Clark et al. (2013) also found that U.S. accessions of *R. armeniacus* clustered with those of German origin in a molecular study of invasive U.S. blackberries.

Conclusions. Important new perspectives developed concerning identity of invasive *Rubus* along the North American Pacific coast, the importance of *R. praecox* in Oregon, and the differential response of these species to a blackberry rust disease.

Table 5. Petal measurements (mm) from *Rubus armeniacus*, *R. praecox* s. lat., and *R. vestitus* from the present study compared with data from the literature.

Rubus sp.	Length × width[a]	Reference
armeniacus	12–17.5 × 10.0–14.5	This study
armeniacus	15–20	Zielinski (2004)
armeniacus	14–20	Weber (1972, 1995)
armeniacus[b]	18 × 13	Edees and Newton (1988)
praecox s. lat.	10–14.5 × 7.2–11.0	This study
praecox	10–13 (-14)	Zielinski (2004)
praecox	10–13 (-14)	Weber (1995)
anglocandicans	13–19 × (9-) 10–13	Evans et al. (2007)
anglocandicans	10 × 7	Edees and Newton (1988)
vestitus	13.0–14.0 × 10.0–12.0	This study
vestitus	10–15	Weber (1972, 1995)
vestitus	9–17 × 6–11	Evans et al. (2007)
vestitus	10–13 ×7–9	Edees and Newton (1988)

[a] Entries showing only a range are about petal length. Outliers in dimensions are set off by parentheses.

[b] Identified as *R. procerus* in the work cited.

Clark et al. (2013) reported that *R. armeniacus* was the more common of the two species in their survey of California, Oregon, and Washington, but in the present study, *R. armeniacus* and *R. praecox* appeared essentially equal in proportion (Table 1; Figure 1). Shift in the predominance of one *Rubus* species over another was reported previously by Bammi and Olmo (1966), who described a shift from the predominance by *R. laciniatus* in the early 1930s to that of *R. procerus* (= either *R. armeniacus* or *R. praecox*) by the 1960s. Results of this study raise a question about the relative importance of *R. armeniacus* vs. *R. praecox* and which will eventually predominate along the West Coast of North America.

A question also arises about effect of the rust disease as an environmental stress factor. Will disease tip the balance in favor of *R. armenicacus* as the species that predominates? Research in the future can examine the importance and role of each of these species as invasives and determine the effect of rust disease on the density of each.

Although separation of *R. armeniacus* from *R. praecox* in the field was generally clear, morphological variants were encountered that were difficult to delimit. This raises the possibility for additional species, not yet recognized, and substantiates the need for additional tools that facilitate blackberry identification. The possibility that variability is related to some sort of crossing event, or hybridization, has been described (Alice 2002; Alice and Campbell 1999), and the importance of hybridization, including development of commercially important varieties within *Rubus*, was discussed by Waugh et al. (1990). Clark and Jasieniuk (2012) more recently presented evidence for natural crosses between native *Rubus ursinus* Cham. & Schldl. × *Rubus pensilvanicus* Poir., introduced into California from the eastern United States, and *R. ursinus* × invasive, exotic *R. armeniacus*. Additional support for naturally occurring hybridization comes from investigation of a blackberry that was intermediate in form between *R. laciniatus* and "*R. procerus*" (Bammi and Olmo 1966).

Species identification and effects of disease on invasive blackberries need to be included in broader ecological studies. Previous findings about blackberry and ecosystem effects can be substantiated or improved, including, e.g., a study on population demographics as affected by growth and reproduction of native and noninvasive *Rubus* species (Lambreche-McDowell and Radosevich 2005); effects of light and soil on blackberry growth, studied earlier for *R. armeniacus* by Caplan and Yeakley (2006); and functional morphology of invasive blackberries and noninvasive species, described also by Caplan and Yeakley (2013) for *R. armeniacus*.

Acknowledgments

Technical support was provided by Sara Meyers and Alyssa Kloos. Reviews and assistance with graphics by colleagues at the USDA, ARS, FDWSRU, is gratefully acknowledged. Christine Dieckhoff (formerly USDA, ARS, Newark, DE) generously made translation from the German of *Rubus* keys by Weber and Mary Camp (USDA, ARS, Beltsville, MD) provided statistical advice and computations. Generous support in the form of consultation, plant accessions (healthy and diseased), and encouragement was provided by the Oregon Department of Agriculture, Salem, OR (Tim Butler, Eric Coombs, Carri Pirosko, Amy Peters, Ken French); by Mark Wiederlechner (Iowa State University, Ames); Jean Shepard (University of California, Davis, Herbarium); Lindsay Clark (University of Illinois, Urbana–Champaign); Gerry Moore (USDA, Natural Resources Conservation Service); and Walt Mahaffee (USDA, ARS, Corvallis, OR). Support was also provided through an internal grant from Palacký University, Olomouc, Czech Republic (IGA_PrF_2014-001, IGA_PrF_2015_001) and Grant No. LO1204 (Sustainable development of research in the Centre of the Region Haná) from the National Program of Sustainability I, Ministry of Education, Youth and Sports of the Czech Republic. Scans of herbarium specimens were provided by Martina Oulehlová, Palacký University, Olomouc, Czech Republic. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Supplementary materials

To view supplementary material for this article, please visit https://doi.org/10.1017/inp.2017.12

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Received September 15, 2016, and approved February 28, 2017.

Associate Editor for this paper: Darren J. Kriticos, CSIRO.