Evaluation of Roses from the Earth-Kind® Trials: Black Spot (*Diplocarpon rosae* Wolf) Resistance and Ploidy

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Abstract. Regional, replicated cultivar trials of landscape roses are an ongoing component of the Earth-Kind® program, which was started at Texas A&M University in the 1990s to support environmental landscape stewardship. The rose trials within the Earth-Kind program identify and promote the most regionally adapted rose cultivars and are conducted without fertilizers or pesticides and greatly reduced irrigation. Black spot (caused by *Diplocarpon rosae* Wolf) is the most serious disease of outdoor-grown roses worldwide as a result of the potential for rapid leaf yellowing and defoliation. Earth-Kind designated cultivars for the south-central United States and roses under trial in other regions or considered for future Earth-Kind trials (n = 73 roses) and two susceptible control cultivars were challenged with North American Races 3, 8, and 9 of *D. rosae*, which were previously characterized at the University of Minnesota. Young expanded leaves were inoculated using detached leaf assays. Lesion length (LL) was measured for susceptible reactions and cultivar ploidy was determined using root tip squashes. Diploid, triploid, and tetraploid cultivars (n = 20, 30, and 23, respectively) were identified, and race-specific resistances and partial resistances were also identified. Race-specific resistance was generally more prevalent in newer rose cultivars and rose cultivars more recently included in Earth-Kind trials. Nine cultivars were resistant to all three races (Brite Eyes™, ‘Grouse’, Home Run®, Knock Out®, Paprika™, Peachy Cream™, Pink Knock Out®, Rainbow Knock Out®, and Yellow Submarine™). Blushing Knock Out®, a sport of Knock Out®, was susceptible to Race 8. Partial resistance rank for LL was generally consistent across races for roses susceptible to multiple races. The application of these data includes: characterizing the minimum resistance level needed for roses to warrant inclusion in Earth-Kind field trials, the identification of additional race-specific resistance genes, identifying resistance-breaking isolates of *D. rosae*, understanding race composition in field trials based on infection patterns of key cultivars, selection of parents for resistance breeding efforts, and continued comparisons between LL and growing bodies of Earth-Kind field resistance data.

Garden roses (*Rosa* sp.) are among the most popular flowering shrubs in the world. Diversity for traits such as form, color, and fragrance of flowers, plant habit, size, environmental adaptability, and extended season of flowering all contribute to their widespread cultivation and versatility (Zlesak, 2006). Lower maintenance cultivars that can tolerate regional environmental conditions without routine dependence on pesticides and excessive care are especially increasing in popularity (Harp et al., 2009; Lonnee, 2005). Factors fueling this trend include negative consumer attitude

toward pesticides, emerging legislation putting greater limits on pesticide availability and use, busy lifestyles, and greater availability of lower maintenance rose cultivars (Harp et al., 2009).

Earth-Kind® Rose Trials are a component of the overall Earth-Kind program, started at Texas A&M (the term Earth-Kind® and associated logo are trademarks of the Texas AgriLife Extension Service, Texas A&M System) and help to serve the horticulture community by identifying the most adaptable landscape roses through regional cultivar trials (Harp

et al., 2009). Pesticides are not used during the trials and cultural management practices use techniques that support environmental stewardship. This includes pre-plant incorporation of compost, maintenance of a 7.6- to 10.2-cm layer of organic mulch, and irrigation methods that conserve water. Before a rose can earn regional Earth-Kind designation, it must exhibit consistent, superior performance across multiyear and multilocation trials representing different soil types and other environmental conditions typical in the region. A large number of cultivars are advertised as low maintenance by nurseries wanting to capitalize on the popularity of lower maintenance roses. It is difficult for consumers to know which roses truly possess the highest levels of pest and environmental tolerances. Earth-Kind designation gives consumers confidence that they are choosing roses that will have a high likelihood of success when basic plant care is provided.

Disease susceptibility poses a major challenge to roses and limits their success as low-maintenance landscape shrubs. Fungi that attack roses include *Diplocarpon rosae* (Wolf) (causal agent of black spot), *Podosphaera pannosa* (Wallr.: Fr.) de Bary (causal agent of powdery mildew), and *Cercospora purideri* B.H. Davis (one of the causal agents of rose leaf spot) (Horst and Cloyd, 2007). Of these, black spot is the most serious in the outdoor landscape across most regions as a result of the potential for rapid disease development that typically leads to leaf yellowing and defoliation (Dobbs, 1984). Black spot has been the most prevalent and widespread disease in the Earth-Kind rose trials (Mackay et al., 2008). Plants repeatedly defoliated from black spot become weakened and quickly fall out of contention for Earth-Kind designation.

Diplocarpon rosae is capable of infecting only the genus *Rosa*. Asexual spores (conidia) overwinter on stems and fallen leaves and are transported to new growth in the spring through water droplets. If free water remains present, the conidia form germ tubes that penetrate the leaf epidermis. Lesions may appear in as little as 4 d as sub-cuticular mycelia radiate from the point of infection. Conidia-bearing acervuli burst through the leaf cuticle followed by leaf abscission in susceptible cultivars (Horst and Cloyd, 2007).

Multiple studies have been conducted to characterize the pathogenic race structure of *D. rosae* and then to use the characterized races to identify genes conferring host resistance (Debener et al., 1998; Whitaker et al., 2007a, 2007b, 2010a; Yokoya et al., 2000). Isolates are distinguished from one another based on their differential ability to infect a common set of rose genotypes. Those isolates with the same host infection pattern are designated as a race. Collections that have been preserved for continued research include six races discovered in Germany (Debener et al., 1998), four from Great Britain (Yokoya et al., 2000), and three races from a genetically diverse set of 50 isolates originating in eastern North America (Whitaker et al., 2007a, 2007b). Whitaker et al. (2010b) evaluated

this international collection, identified 11 unique races among them, and standardized the race nomenclature. These studies and others have uncovered genetic resistance within rose species and cultivars that is race-specific. Such race-specific resistance can only be characterized with controlled inoculations of roses with individual races as opposed to a field setting where the presence and prevalence of specific races are not known. Using characterized races for inoculations under controlled conditions is also advantageous when surveying for partial resistance (Whitaker and Hokanson, 2009a; Xue and Davidson, 1998).

Detached leaf assays have been an efficient tool to characterize the resistance of rose seedling populations, and they have been found to be strongly correlated with whole plant inoculations (Hattendorf et al., 2004; Von Malek and Debener, 1998; Whitaker and Hokanson, 2009a). Detached leaf assays are preferable as a result of greater ease in controlling humidity and inoculum levels (Whitaker and Hokanson, 2009b). To the best of our knowledge, single-spore isolates of *D. rosae* have only been used for race characterization, comparison of pathogenicity of isolates, and to study the segregation of resistance to particular races in genetic studies and the characterization of partial resistance components. Single-spore isolates representing different races to the best of our knowledge have not been used to individually challenge commercial cultivars for widespread race-specific and horizontal resistance characterization. Schulz et al. (2009) challenged rose accessions uninfected with black spot in two field locations with a mixture of single-spore *D. rosae* isolates using detached leaf assays. However, the number of races represented by these isolates is not known. Recently, Whitaker and Hokanson (2009a) reported using detached leaf assays to characterize the partial resistance to black spot of segregating populations using the measurement of LL, defined as the diameter of the lesion at its widest point. LL was chosen by the authors because of the ease of measurement and its significant correlations with three other measures of partial resistance (Whitaker et al., 2007b; Xue and Davidson, 1998).

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Using a collection of races to characterize roses marketed for low-maintenance landscape use would be helpful beyond highlighting the resistance of roses for growers and consumers. Knowledge of the genetic resistance of these roses would guide the selection of cultivars that could serve as controls to help differentiate the presence of known races in field trials where race composition is not known. Additionally, when cultivars with resistance to all known races become infected, pathologists may characterize the infective isolate(s) in search of new pathogenic races.

A widespread cultivar screen with known races would also allow breeders to identify desirable genetic resistances among a core set of highly resistant cultivars. If knowledge of resistance could be coupled with knowledge of rose ploidy level, this would help breeders develop disease-resistant cultivars more efficiently because of a greater likelihood of reproductive success. Preferential crossability and fertility as a consequence of ploidy level has been well documented in roses (El Mokadem et al., 2001; Leus, 2005; Rowley, 1960; Shahare and Shastri, 1963).

The objectives of this study were to characterize roses within or being considered for the Earth-Kind rose program according to 1) resistance to three North American races of *D. rosae* using detached leaf assays; and 2) ploidy through direct chromosome counts.

Materials and Methods

Plant material. Seventy-five rose cultivars were challenged for race-specific and partial resistance and characterized for ploidy (Table 1). These roses represent 17 Earth-Kind-designated cultivars for the south-central United States (the only region currently where roses have completed the Earth-Kind trialing process and winners are designated), 30 cultivars under trial in the Earth-Kind Brigade (mid-U.S. Earth-Kind rose trials), 20 cultivars under trial in the Northern Earth-Kind Rose Trials (north-central U.S. Earth-Kind rose trials; Harp et al., 2009), and two controls. A limited number of cultivars are represented in multiple Earth-Kind regional groups such as Carefree Beauty™ ('Bucbi') and 'Sea Foam', which are in all three groups. In addition, roses were included that are developing reputations as being highly resistant to black spot and are being considered for future Earth-Kind trials (Alba Meidiland™, Candy Oh™ Vivid Red, Carefree Celebration™, Carefree Marvel™, Double Knock Out®, Fragrant Spreader™, Home Run®, Paprika™, Peachy Cream™, Rainbow Knock Out®, Strawberry Crush™, and Sunny Knock Out®). Also included are parents or offspring of very resistant roses ('Grouse' and 'Plaisanterie'). The two susceptible controls were 'Chorale' and 'Pariser Charme'.

At least two potted plants of each cultivar were acquired from multiple nursery sources and plants were all propagated on their own roots (not grafted). Own root plants were necessary to allow for cultivar ploidy determination using root tip squashes. Potted plants

of cultivars were obtained in the summer of 2007 and maintained on an outdoor gravel pad. They were transferred to a cold room and provided with a 4 °C vernalization treatment for ≈10 weeks before being pruned, repotted as necessary, and brought to the greenhouse (23 ± 4.0 °C; St. Paul, MN; lat. 45° N) in mid-Jan. 2008. Day extension with supplemental light was used until 2200 HR (400-W metal halide lamps; ≈150 mmol·m⁻²·s⁻¹ at plant level) to encourage strong, vigorous growth. Sulfur was burned in the greenhouse from 22:00 to 02:00 HR nightly to discourage powdery mildew. Sulfur burners were turned off a minimum of 3 d before leaves were collected for detached leaf assays.

In late May 2008, a subset of roses needing further characterization as a result of missing data or to better understand inconsistent results was given an ≈8-week vernalization (4 °C) treatment to reinvigorate growth. After vernalization, roses were cut back, repotted as needed, and brought to the St. Paul greenhouse in mid-July and grown as described previously. There was a final, yet smaller, subset of roses forced for the same reasons. They were provided with a ≈12-week vernalization treatment in mid-Oct. 2008 (4 °C; the longer vernalization treatment was the result of greenhouse availability and logistics) and brought to the University of Minnesota Horticulture Research Center greenhouse in Chanhassen, MN, in mid-Jan. 2009 and grown under similar conditions as previously described.

Isolate preparation and inoculations. Fungal isolates ACT (Race 8; collected from Brenham, TX), GVH (Race 3; collected from Hastings, MN), and IGWA (Race 9; collected from Appleton, WI) described in Whitaker et al. (2010b) were retrieved from liquid nitrogen storage. To obtain sufficient inoculum, aqueous suspensions of asexual spores (conidia) were pipetted onto leaves of susceptible cultivars. Leaves were placed adaxial side up on a folded 15 × 28-cm sheet of paper towel (Bounty®; Proctor and Gamble, Cincinnati, OH) moistened with distilled water and sealed in an airtight, transparent Clearpac® deli container (22.9 cm length × 18.7 cm width × 6 cm height; cat. # C48DER; Dart Corporation, Mason, MI). Containers (subsequently referred to as boxes) were incubated in the laboratory at room temperature (21 to 24 °C). After the development of sporulating lesions in 10 to 14 d, the infected leaves were stored in sealed polyethylene bags at -20 °C for up to 6 weeks before inoculation. Spores from the frozen leaves were collected by washing the lesions with distilled, deionized water and quantified using a hemacytometer.

The two primary inoculation experiments were performed on 28 Feb. 2008 and 3 Apr. 2008. The third, fourth, and fifth inoculations were performed on 3 Aug. and 9 Sept. 2008 and 11 Mar. 2009, respectively. These additional inoculations over time were performed for those host/isolate combinations having missing data resulting from leaf degradation or inconsistent results across the first two inoculation dates. A minimum of four inoculation boxes of leaves and a maximum of 10 were

Table 1. Rose cultivars with their Earth-Kind group classification, commercial class, year of introduction, ploidy, and *Diplocarpon rosae* race-specific reaction.

Cultivar ^a	Category ^a	Commercial class	Yr of introduction and breeder ^w	Ploidy	Race 3	Race 8	Race 9
Alba Meidiland™ (MEIflopan)	4	Shrub	1985 ¹	2x	S ^z	S*	S*
Alexander Mackenzie	3	Shrub	1985 ²	3x	S	R	R
Amiga Mia	2	Shrub	1978 ³	4x	S	S	S*
April Moon	2	Shrub	1984 ³	3x	S	S	S
Barn Dance	2	Shrub	1975 ³	3x	R	S	R
Belindas Dream	1, 2	Shrub	1992 ⁴	3x	S	S	S
Blushing Knock Out® (RADyod)	2	Shrub	2004 ⁵	3x	R	S	R
Brite Eyes™ (RADbrit)	3	Large-flowered climber	2006 ⁵	4x	R	R	R
Caldwell Pink	1	Polyantha	Unknown ⁶	2x	S	R	R
Candy Oh™ Vivid Red (ZLEmartincipar)	4	Shrub	2008 ⁷	2x	S	S	R
Carefree Beauty™ (BUCbi)	1, 2, 3	Shrub	1977 ³	4x	S	S	S
Carefree Celebration™ (RADral)	4	Shrub	2008 ⁵	3x	S	S	S*
Carefree Marvel™ (MEIrameca)	4	Shrub	2003 ⁸	3x	S	S	R
Carefree Wonder™ (MEIpitac)	2	Shrub	1990 ⁸	4x	S	S	S
Chorale	5	Shrub	1978 ³	4x	S	S	S
Chuckles	2	Floribunda	1958 ⁹	4x	S	S	S
Climbing Pinkie	1	Climbing polyantha	1952 ¹⁰	2x	S	S	S
Country Dancer	2	Shrub	1973 ³	4x	S	S	R
Double Knock Out® (RADtko)	4	Shrub	2004 ⁵	3x	R	S*	R
Dublin Bay™ (MACdub)	2	Climbing floribunda	1975 ¹¹	4x	S	S	S
Ducher	1	China	1869 ¹²	2x	S	S	S
Duchesse de Brabant	1	Tea	1857 ¹³	2x	S	S	S
Earth Song	2	Shrub	1975 ³	4x	S	S	S
Else Poulsen	1	Floribunda	1924 ¹⁴	3x	S	S	S
Flora Dora	2	Shrub	Unknown ⁶	3x	S	S	S*
Folksinger	2	Shrub	1985 ³	4x	S	R	S
Fragrant Spreader™ (CHEWground)	4	Shrub	2002 ¹⁵	2x	S*	S	R
Frontenac	3	Shrub	1992 ²	4x	S	R	R
Georgetown Tea	1	Tea	Unknown ⁶	2x	S	S	S
George Vancouver	3	Shrub	1994 ²	4x	S	R	S
Grouse™ (KORimro)	4	Shrub	1982 ¹⁶	2x	R	R	R
John Cabot	3	Hybrid kordesii	1978 ²	4x	S	S	S
John Davis	3	Hybrid kordesii	1986 ²	3x	S*	S*	R
Home Run® (WEKcisbako)	4	Shrub	2006 ¹⁷	3x	R	R	R
Knock Out® (RADrazz)	1, 2	Shrub	1999 ⁵	3x	R	R	R
Lena (BAIlena)	3	Shrub	2007 ¹⁸	2x	S	S	S
Marie Daly	1	Polyantha	Unknown ⁶	2x	S	S	S
Mme. Antoine Mari	1	Tea	1901 ¹⁹	2x	S	S	S
Morden Blush	3	Shrub	1988 ²⁰	4x	S	S	S
Mutabilis	1	China	<1894 ⁶	2x	S	S	S
New Dawn	1, 2	Large-flowered climber	1930 ²¹	3x	S	S	S*
Ole (BAIole)	3	Shrub	2007 ¹⁸	2x	S	S	S
Paprika™ (CHEWmaytime)	4	Shrub	2006 ¹⁵	3x	R	R	R
Pariser Charme	5	Floribunda	1965 ²²	3x	S	S*	S
Peachy Cream™ (HORcoherent)	4	Shrub	2003 ²³	3x	R	R	R
Pearlie Mae	2	Shrub	1981 ³	3x	S	S	S
Penelope	2	Hybrid musk	1924 ²⁴	3x	S	S	S
Perle d'Or	1	Polyantha	1884 ²⁵	2x	S	S*	R
Pink Knock Out® (RADcon)	2	Shrub	2005 ⁵	3x	R	R	R
Plaisanterie™ (LENTrimera)	4	Hybrid musk	1996 ²⁶	2x	S	S	S
Polar Joy™ (BAIore)	3	Shrub	2004 ²⁷	3x	S	R	R
Polonaise	2	Shrub	1984 ³	3x	S	S	S
Prairie Breeze	2	Shrub	1978 ³	4x	S	S	S
Prairie Harvest	2	Shrub	1985 ³	3x	S	R	S
Prairie Joy	3	Shrub	1990 ²⁸	3x	S	S	S
Prairie Princess	2	Shrub	1972 ³	4x	S	S	S
Princess Verona	2	Shrub	1984 ³	3x	S	S	S
Quadra	3	Hybrid kordesii	1994 ²	4x	S	R	R
Quietness	2	Shrub	2003 ³	3x	S	R	R
Rainbow Knock Out® (RADcor)	4	Shrub	2007 ⁵	3x	R	R	R
Ramblin Red	3	Large-flowered climber	2001 ⁵	4x	S	R	R
Rosarium Uetersen™ (KORtersen)	2	Large-flowered climber	1977 ¹⁶	4x	S	S	S
Sea Foam	1, 2, 3	Shrub	1964 ²⁹	3x	S	S	S
Spice	1	China	Unknown ⁶	2x	S	S	S
Square Dancer	2	Shrub	1972 ³	4x	S	R	R
Strawberry Crush™ (HORMeteorie)	4	Shrub	2008 ²³	3x	S	S	S
Summer Wind	2, 3	Shrub	1975 ³	4x	S	S	R
Sunny Knock Out® (RADsunny)	4	Shrub	2008 ⁵	3x	S	R	S
Sunrise Sunset™ (BAIset)	3	Shrub	2004 ²⁷	3x	S	S	S*
Sven (BAISven)	3	Shrub	2007 ¹⁸	2x	S	S	S
The Fairy	1, 2	Polyantha	1932 ³⁰	2x	S	S	S
The Gift	4	Polyantha	1981 ³¹	2x	S	S	S*
William Baffin	3	Hybrid kordesii	1983 ³	4x	S	R	R
Winter Sunset	2	Shrub	1997 ³	4x	S	S	R
Yellow Submarine™ (BAIline)	3	Shrub	2004 ²⁷	4x	R	R	R

^zS = susceptible; R = resistant; S* = more than one but less than half of the replicates had susceptible reactions.^aCultivar name or widely known trademark name is listed followed by registered cultivar name, if different.¹ = Earth-Kind-designated rose for the south-central United States; 2 = Earth-Kind Brigade; 3 = Northern Earth-Kind Rose Trials; 4 = under consideration for future Earth-Kind rose trials; 5 = susceptible control cultivars.^wBreeder or discoverer: ¹Marie-Louise Meilland; ²Felicitas Svejdá; ³Griffith Buck; ⁴Robert Basye; ⁵William Radler; ⁶Unknown; ⁷David Zlesak; ⁸Alain Meilland; ⁹Roy Shepherd; ¹⁰E.P. Dering; ¹¹Samuel McGredy IV; ¹²Jean-Claude Ducher; ¹³H. Bernède; ¹⁴Svend Poulsen; ¹⁵Christopher Warner; ¹⁶Reimer Kordes; ¹⁷Tom Carruth; ¹⁸Kathy Zuzek; ¹⁹Antoine Mari; ²⁰Lynn Collicutt; ²¹Henry Bosenberg; ²²Mathias Tantau, Jr.; ²³Colin Horner; ²⁴Joseph Pemberton; ²⁵Joseph Rambaux; ²⁶Louis Lens; ²⁷Ping Lim; ²⁸Henry Marshall; ²⁹Ernest Schwartz; ³⁰Ann Bental; ³¹Joyce Demits.^v'Pariser Charme' was used as a susceptible substitute control cultivar in this study for one inoculation over time for Races 3 and 9 as a result of its previously characterized susceptibility to these races (Whitaker et al., 2010b).

prepared for each cultivar × race combination over the course of this study. For each cultivar × race combination assessed at an inoculation date, two boxes of leaves were prepared. ‘Chorale’ was the universal susceptible control cultivar for all inoculations except for 9 Sept. 2008 when it was not available in sufficient quantity and ‘Pariser Charme’, also susceptible to all three races (Whitaker and Hokanson, 2009b), was substituted.

Conidial concentrations of the inoculum varied with availability on each date and ranged between 30,000 and 100,000 conidia/mL. These concentrations were within the range of inoculum concentrations used in other studies (Whitaker et al., 2007b, 2010b; Whitaker and Hokanson, 2009a). At each of the five inoculation dates one susceptible control cultivar was used. A limited group of cultivars in this study were previously characterized for resistance to these same races (‘Folksinger’, ‘John Cabot’, ‘Lena’, ‘Morden Blush’, ‘Ole’, ‘Sea Foam’, and ‘Sven’) and served as additional, internal controls (Hokanson et al., 2007; Whitaker and Hokanson, 2009a; Whitaker et al., 2007b). A replicate consisted of two or three young fully expanded leaves (each composed of three to seven leaflets) placed adaxial side up on a moistened paper towel within a box as described previously. A handheld spray bottle was calibrated to deliver 0.75 mL of inoculum per spray, and each box was sprayed twice (1.5 mL per box). After 48 h, the boxes were briefly opened to blot off inoculum droplets from the leaf surfaces.

In a rare instance, detached leaves of Polar Joy™ (‘BALore’) repeatedly deteriorated within 2 to 3 d after inoculation. To understand the race resistance of this cultivar, attached leaf inoculations were conducted according to Whitaker et al. (2007b). Young fully expanded leaves were sprayed with the same inoculum preparations as for detached leaf assays. Plants were brought to the laboratory for inoculation so that ambient temperatures were the same for the attached leaves and the detached leaves. Inoculated leaves of Polar Joy™ were individually sealed in plastic bags to keep humidity high for 48 h. Bags were removed and the plants were brought back to the greenhouse where resistance or susceptibility was noted on the same day as the detached leaves inoculated at the same time.

Disease rating. Ratings were performed 12 to 16 d post-inoculation with the exact number of days before rating per inoculation over time dependent on the progression of disease. Replicates inoculated on a common date were also rated on a common date. Leaves with lesions that contained spore-bearing acervuli were rated as susceptible. For susceptible reactions, the partial resistance component of LL (see Xue and Davidson, 1998) was evaluated by measuring the diameter of the largest lesion on each leaf using a digital caliper. Leaves bearing lesions with no or few acervuli during the first rating were incubated for 2 more days and examined again for the presence of acervuli using a 40× dissecting microscope, although LL was not reassessed at that time.

Ploidy assessment. Root tip squashes were used to isolate cells in metaphase for chromosome counts for rose cultivars in which ploidy level was not already determined by this direct method (Zlesak, 2009). Chromosomes of five or more well-spread metaphase cells were observed and counted per genotype. Ploidy level was determined by dividing the number of observed chromosomes by seven (for roses $x = 7$). Actively growing root tips were harvested from potted roses in the greenhouse and stored in vials of water on ice for up to 24 h. Root tips were subsequently fixed in Farmer’s fixative [3:1 (v/v), 95% ethanol: glacial acetic acid] and refrigerated until the day of observation. Water was replaced with 6 N HCl for hydrolyzation of cells for 90 min at room temperature, just before squashing, and acetocarmine was used for staining.

Statistical analysis. Lesion length data for susceptible cultivars were analyzed independently for each race using analysis of variance (ANOVA) to compare factors and to compare infected cultivars for relative partial resistance. The mean LL was calculated for each inoculation box and that single value was used for the analyses. The factors in the ANOVA were: cultivar, time of inoculation, and the cultivar × time interaction. Mean separations were performed using Tukey’s honestly significant difference (HSD) ($P \leq 0.05$). ANOVA was also calculated for roses clearly susceptible to all races (50% or more boxes were infected) with the following factors: cultivar, race, time, and their interactions. Pearson’s correlation was calculated between mean LL (across all infected boxes) and mean overall landscape performance rating (Mackay et al., 2008) and mean LL and the 2-year mean black spot field defoliation rating (Colbaugh et al., 2005) for south-central U.S. Earth-Kind winning roses. The landscape performance data and defoliation data were taken at a common trial site in Dallas, TX (Colbaugh et al., 2005; Mackay et al., 2008). All statistics were computed using SPSS 12.0 software (SPSS Inc., Chicago, IL).

Results

Race-specific resistance was found among rose cultivars within the Earth-Kind program to Races 3, 8, and 9 of *D. rosae* (Table 1). Of the eight possible race-specific resistant/susceptible classes for the three races, six were represented (Table 2). Nine roses were not infected by any of the three races (Brite Eyes™, ‘Grouse’, Home Run®, Knock Out®, Paprika™, Peachy Cream™, Pink Knock Out®, Rainbow Knock Out®, and Yellow Submarine™). Twelve roses were resistant to two pathogenic races and 11 cultivars were resistant to only one pathogenic race. The majority of roses ($n = 41$), however, were susceptible to all three races.

Determining race-specific resistance of a limited group of cultivars was challenging as a result of inconsistent infection patterns, which was likely complicated by high partial resistance that delayed or prevented the

Table 2. Number of cultivars in the Earth-Kind program (not including controls) in each of the eight possible combinations for resistant (R) and susceptible (S) reactions for the three races of *Diplocarpon rosae*.

No. of cultivars	Percent cultivars	Race 3	Race 8	Race 9
9	11.1	R	R	R
9	12.5	S	R	R
0	0.0	R	R	S
4	5.6	S	R	S
3	4.2	R	S	R
7	9.7	S	S	R
0	0.0	R	S	S
41	56.9	S	S	S

formation of acervuli. Some roses had infection in only a single or small subset of inoculated boxes. When this occurred, additional boxes beyond the original four (minimum of six and up to 10 total) were inoculated with the race in question over additional inoculation dates. If leaves in only one box produced a sporulating lesion out of the six (or more) boxes inoculated, the cultivar was rated as resistant to that race. If more than one but less than half of the boxes contained infected leaves, the rose was classified as susceptible and is designated with an asterisk in Table 1. There were 13 susceptible rose/race combinations out of the 159 total susceptible rose/race combinations using detached leaf assays in which more than one but less than half of the boxes had infected leaves. Typically, susceptible cultivar/race combinations resulted in leaves in all boxes being infected.

The roses that were previously characterized for their resistance to these three races displayed race-specific infection patterns in this study that were consistent with previous results (‘Folksinger’, ‘John Cabot’, ‘Lena’, ‘Morden Blush’, ‘Ole’, ‘Sea Foam’, and ‘Sven’; Hokanson et al., 2007; Whitaker et al., 2007b; Whitaker and Hokanson, 2009a).

Polar Joy™ (‘BALore’) was unique in that its detached leaves consistently deteriorated after 2 to 3 d of incubation. Leaves of Polar Joy™ differed from those of other cultivars in that they were noticeably pubescent. Inoculations of intact leaves were performed as described in the “Materials and Methods.” General race-specific reactions were recorded on the same date as the detached leaf assays inoculated at the same time and reactions were consistent across inoculations (susceptible to Race 3 only). LL data were not gathered for comparison with other cultivars susceptible to Race 3 because of the altered infection and incubation environments.

Variation was found for lesion size among susceptible cultivar/race combinations, indicating variability for partial resistance (Tables 3 and 4). Separate ANOVAs were performed for each race (Table 3). Cultivars are ranked based on mean lesion size for each race in Table 4 with significance groups highlighted (based on Tukey’s HSD, $P \leq 0.05$). Generally, roses susceptible to multiple races tended to have similar rank positions. For instance, Alba Meidiland™ (‘Meilfopan’) was susceptible to all three races and was among rose cultivars

Table 3. Mean squares from analysis of variance for effects resulting from cultivar (susceptible only), time of inoculation, and their interaction on lesion length calculated for *Diplocarpon rosae* Races 3, 8, and 9.

Factors	Race 3	Race 8	Race 9
Cultivar	2.48**	2.05**	1.98**
Time	6.58**	6.34**	2.36**
Cultivar × time	1.01**	0.43*	0.81**
Error	0.53	0.29	0.33

**Significant at $P = 0.01$; *significant at $P = 0.05$.

within the 15% smallest LL for all three races. ‘Belinda’s Dream’, on the other hand, was consistently among rose cultivars with the 15% largest LL for all three races. Strikingly, ‘The Fairy’ and ‘Lena’ ranked first and second across all three races for smallest LL.

There were 34 roses susceptible to all three races with 50% or more of the boxes containing infected leaves. The mean squares from ANOVA and their significance for the factors cultivar, race, time of inoculation, and their interactions are presented in Table 5. All factors and interactions were significant at $P \leq 0.01$, except for cultivar × race, which was significant at $P \leq 0.05$, and the race × time and cultivar × race × time interactions, which were not significant.

A trend was observed for the prevalence of race-specific resistance across the four different groups of cultivars within the Earth-Kind program. The groups differ based on how long the cultivars have been in the program such that the longer they have been in the program, the fewer race-specific resistance reactions were observed. Cultivars that have been in the program the longest are those that have earned Earth-Kind designation in the south-central United States. This group displayed the fewest number of cultivars with race-specific resistance (18%) followed by the Earth-Kind Brigade (37%), the Northern Earth-Kind Rose Trials (50%), and finally roses entering or being considered for Earth-Kind trialing (64%).

For the 17 south-central U.S. Earth-Kind-designated roses, overall mean performance ratings over a 3-year field evaluation trial in Dallas, TX, have been reported (Mackay et al., 2008) as well as defoliation ratings on the same plants over 2 years (Colbaugh et al., 2005). The landscape performance scale ranged from 0 to 10 with 10 being best and the defoliation scale ranged from 0 to 5 with 5 being completely defoliated. The Pearson’s correlation value between mean LL (averaged over races and boxes) and overall performance rating had a significant, inverse correlation ($r = -0.642$; $P < 0.01$) and LL and overall 2-year mean defoliation rating had a significant, positive correlation ($r = 0.618$; $P = 0.01$). Therefore, larger LLs were associated with lower mean overall performance ratings and larger LLs were associated with greater plant defoliation. Knock Out® was omitted from the correlations because LL was not available because it was not infected by any of these three races.

The breeding programs with the most roses represented in this study include those led by the late Dr. Griffith Buck at Iowa State

University in Ames, IA (17 cultivars; marketed as Buck roses) and by Dr. Felicitas Svedja from Agriculture Canada in Ottawa, Ontario (seven cultivars; marketed as Explorer™ roses). Trends were not detected among the Buck roses for race resistance. There was documented susceptibility and resistance across roses for each of the races and no clear pattern of susceptibility or resistance based on introduction date. All seven of the Explorer™ cultivars, on the other hand, were susceptible to Race 3, whereas resistances to Races 8 and 9 were variable and did not appear to be associated with introduction date.

Chromosome counts revealed that most ($n = 30$) roses were triploid (Table 1). In addition, half of the 10 commercial classes of roses were represented by multiple ploidy levels (Table 6). After triploid, the next most prevalent ploidy level was tetraploid and lastly diploid ($n = 23$ and 20 , respectively; Table 6). One susceptible control was tetraploid (‘Chorale’) and the other was triploid (‘Pariser Charme’).

Discussion

In this study, race-specific resistances were discovered that were consistent among replications and with previous results (Hokanson et al., 2007; Whitaker et al., 2007b; Whitaker and Hokanson, 2009a). Moreover, differences in partial resistance were discovered among susceptible genotypes and relative rank was generally consistent for cultivars susceptible to multiple races. Comparing results from the present study with results of previous Earth-Kind field trials indicates that data generated from laboratory-detached leaf assays are significantly correlated with both field defoliation as a result of black spot and overall cultivar performance ratings. Based on these results, further use of this technique in the Earth-Kind program would be warranted to characterize roses entered in the program and to consider using this assay to pre-screen future entrants for inclusion into the program.

Attempting to predict which roses will possess durable black spot resistance is a challenging prospect when relying solely on field observations. Cultivars may display strong field resistance when first released into commerce, leading to their wide distribution. Later, however, they may succumb to new or migrating pathogenic races, especially when they possess low levels of partial resistance. This scenario has been documented with the roses ‘Baby Love’ and ‘Martin Frobisher’ (Bolton and Svejda, 1979; Yokoya et al., 2000). Consumers become confused and disappointed when bold marketing claims surrounding new landscape roses are not realized. In addition, the relative amount of black spot infection on susceptible cultivars can change from season to season in a single planting. Colbaugh et al. (2005) reported defoliation data resulting from black spot for 107 rose cultivars over two growing seasons, and although the overall Pearson correlation across years of their study was significant and positive ($r = 0.627$; $P < 0.01$; calculated from their

reported data), variable relative resistance was found across years for many cultivars.

Our data show a high frequency of race-specific resistance. Race-specific resistances in some cultivars (including ‘Folksinger’ and ‘George Vancouver’, which are included in this study) are controlled by single loci (Whitaker et al., 2010a). Such resistances are not likely to be durable. Mutations in an avirulence gene in the pathogen and/or migration of pathogenic isolates can soon lead to the loss of such resistances. Patterns of genetic diversity of *D. rosae* isolates from eastern North America are consistent with widespread geographical mixing of isolates of *D. rosae*, possibly through the transportation of commercially sold roses (Whitaker et al., 2007a). Using multiple, characterized races of *D. rosae* to challenge landscape roses in controlled settings would be very valuable to help ascertain the relative partial resistance of a cultivar once race-specific resistance(s) have been compromised.

Obtaining a set of *D. rosae* isolates that can overcome race-specific resistance(s) of any cultivar (thereby exposing underlying partial resistance) is now possible. In the host-isolate differential array used to establish international nomenclature for *D. rosae* races, none of the 15 roses used as hosts, including the highly resistant Knock Out® rose, had race-specific resistance to all 11 races (Whitaker et al., 2010b). As more races are identified and added to the international race collection, it will become an even stronger resource for identifying cultivar resistance as well as aiding in the breeding of durably resistant cultivars. This current study serves as an initial effort for widespread screening of cultivars and uses characterized North American *D. rosae* races to aid in describing the resistance of roses in the North American Earth-Kind trials. Future cultivar screens would be possible using a subset of races from the international race collection for even greater applicability (i.e., a subset of races can be chosen that collectively infects all roses challenged to date).

Detached leaf assays are a controlled high-throughput method for characterizing resistance, and LL data from these assays have been positively correlated with whole plant inoculation assays (Jenkins, 1955; Whitaker and Hokanson, 2009a). One of the challenges with controlled inoculations on whole plants or detached leaves is that susceptibility can vary based on leaf age (Horst and Cloyd, 2007). Very young foliage has greater susceptibility than more mature foliage, although in the field, black spot tends to appear first on older foliage as a result of increased humidity within the lower canopy of the plant (Horst and Cloyd, 2007). Although care was taken to try to standardize leaf age in this study by using only recently, fully expanded leaves, leaf maturity likely introduced some variability. Replication of cultivar × race combinations over multiple boxes and over time is important to gain a more complete and accurate understanding of a cultivar’s resistance. When considering LL of only roses susceptible to all three races, the ANOVA reveals the main factors (cultivar, race, and time of

Table 4. Black spot lesion length for rose cultivars susceptible to one or more of Races 3, 8, and 9 of *Diplocarpon rosae*.

Race 3			Race 8			Race 9		
Cultivar	Lesion length (mm)	Significance groups ²	Cultivar	Lesion length (mm)	Significance groups	Cultivar	Lesion length (mm)	Significance groups
The Fairy	1.51		The Fairy	1.22		The Fairy	0.79	
Lena	1.57		Lena	1.44		Lena	1.02	
Candy Oh™	1.65		Sunrise Sunset™	1.47		The Gift	1.13	
Vivid Red	1.87		Alba	1.47		Barn Dance	1.16	
Fragrant Spreader	1.97		Meidiland™	1.47		Polonaise	1.17	
Amiga Mia	1.98		Carefree Celebration™	1.47		Alba	1.18	
The Gift	1.99		Double Knock	1.49		Meidiland™	1.26	
Alba	2.02		Out®	1.52		Prairie Princess	1.32	
Meidiland™	2.04		Carefree Beauty™	1.53		Carefree Beauty™	1.41	
New Dawn	2.06		Amiga Mia	1.54		George	1.43	
Carefree Beauty™	2.10		John Davis	1.70		Vancouver	1.44	
Sea Foam	2.11		Country Dancer	1.77		Sven	1.50	
John Davis	2.12		New Dawn	1.82		Amiga Mia	1.52	
Barn Dance	2.15		Perle d' Or	1.84		New Dawn	1.58	
Sunrise Sunset™	2.18		Summer Wind	1.84		Folksinger	1.58	
Winter Sunset	2.23		Carefree Marvel™	1.91		John Cabot	1.71	
Ole	2.25		Barn Dance	1.95		Prairie Joy	1.72	
Alexander	2.31		Winter Sunset	2.10		Chuckles	1.90	
Mackenzie	2.31		The Gift	2.11		Carefree Celebration™	1.92	
Carefree Marvel™	2.32		Polonaise	2.17		Sunny Knock	1.93	
Prairie Princess	2.38		Candy Oh™	2.18		Out®	1.94	
Carefree Celebration™	2.43		Vivid Red	2.18		Prairie Breeze	2.04	
Sunny Knock	2.56		Flora Dora	2.19		Pearlie Mae	2.05	
Out®	2.56		Spice	2.22		Georgetown Tea	2.06	
William Baffin	2.64		Fragrant Spreader™	2.27		Earth Song	2.11	
Country Dancer	2.74		Prairie Princess	2.28		Ole	2.24	
Sven	2.75		Sea Foam	2.29		Sea Foam	2.27	
Seminole Wind	2.78		Georgetown Tea	2.35		Mutabilis	2.35	
Folksinger	2.88		Seminole Wind	2.42		Flora Dora	2.51	
Frontenac	2.95		Pearlie Mae	2.51		Prairie Harvest	2.54	
Prairie Harvest	2.97		Ole	2.54		Spice	2.54	
Summer Wind	2.98		John Cabot	2.67		Strawberry Crush™	2.56	
Perle d' Or	3.03		Blushing Knock	2.71		Seminole Wind	2.61	
Prairie Joy	3.03		Out®	2.72		Duchesse de Brabant	2.61	
Carefree Wonder™	3.10		Dublin Bay	2.82		Brabant	2.64	
Mutabilis	3.17		Sven	2.82		Dublin Bay	2.73	
Pearlie Mae	3.24		Prairie Joy	2.82		April Moon	2.75	
Earth Song	3.25		Chuckles	2.88		Carefree Wonder™	2.78	
Caldwell Pink	3.27		Morden Blush	2.92		Morden Blush	2.87	
Quadra	3.29		Prairie Breeze	3.00		Sunrise Sunset™	2.89	
Marie Daly	3.37		Carefree Wonder™	3.02		Mme. Antoine	2.96	
Else Poulsen	3.41		Strawberry Crush™	3.12		Mari	2.96	
George	3.42		Ducher	3.14		Penelope	3.14	
Vancouver	3.47		Mutabilis	3.15		Else Poulsen	3.25	
Dublin Bay	3.48		Earth Song	3.28		Climbing Pinkie	3.31	
Chuckles	3.54		Mme. Antoine	3.35		Belinda's Dream	3.37	
Morden Blush	3.55		Mari	3.47		Pariser Charme®	4.14	
Duchesse de Brabant	3.58		April Moon	3.51		Marie Daly	3.14	
Brabant	3.58		Princess Verona	3.56		Ducher	3.25	
Prairie Breeze	3.64		Chorale	3.56		Plaisanterie	3.31	
Flora Dora	3.66		Climbing Pinkie	3.72		Chorale	3.37	
Polonaise	3.68		Penelope	3.72		Princess Verona	3.87	
Spice	3.69		Duchesse de Brabant	3.94		Pariser Charme®	4.14	
Chorale	3.97		Else Poulsen					
Penelope	3.97		Mari					
Plaisanterie	4.04							
Square Dancer	4.10							
John Cabot	4.19							
Climbing Pinkie	4.20							
Strawberry Crush™	4.23							
Quietness	4.23							
Mme. Antoine	4.23							
Mari	4.23							
Georgetown Tea	4.23							
April Moon	4.23							
Ducher	4.23							
Princess Verona	4.23							
Belinda's Dream	4.23							
Ramblin Red™	4.23							
Pariser Charme®	5.17							

²Means within column under race connected by a common vertical line do not differ significantly using Tukey's honestly significant difference ($P = 0.05$).

inoculation) and the two-way interactions were significant ($P \leq 0.05$), except for the race $\times$ time interaction (Table 5). Among other factors, the significance of time of inoculation and its interactions may be the result of leaf maturity differences.

In addition to the potential for very young leaves having atypical susceptibility to a race, strong partial resistance seemed to create difficulty in classification of some roses for race susceptibility (Table 1). Whitaker et al. (2007b) reported difficulty characterizing race specific versus partial resistance for 'Sea Foam' and attribute this to strong partial resistance. Inconsistent results led to added inoculations over time and the distinction within susceptible roses displaying infection in at least two boxes (Table 1) but less than half of the overall boxes (at least six boxes in such incidences were inoculated).

Roses enter the Earth-Kind program because of their reputation for strong performance among nursery and landscape professionals and rose society members (Harp et al., 2009). Cultivars are then planted and evaluated in replicated, randomized Earth-Kind regional field trials and the best performers are awarded with Earth-Kind regional designation. The goal is to designate any deserving rose with Earth-Kind status no matter where it was developed or how long it has been on the market. The trend for rose groups having been in the Earth-Kind program longer to have a lower frequency of cultivars with race-specific resistance is worthy of note. In the process of cultivar development, breeders select the healthiest roses with the least amount of black spot symptoms and do so typically in a relatively short period of time and in a fairly

restricted locale. As such, breeders may be selecting strongly for race-specific resistance. Over longer periods of time and over multiple locations, roses that maintain useful resistance in the landscape and earn Earth-Kind designation seem to primarily be those that possess strong partial resistance.

In this study, nine roses proved resistant to all three races of *D. rosae*. The question may be raised whether these roses possess race-specific resistance or, in a very unlikely possibility, are universally resistant to all races. We are making the assumption that roses resistant to all three races possess race-specific resistance. However, until a single-spore isolate is shown to infect such a rose, race-specific resistance cannot be differentiated from the very unlikely event of universal resistance. Race-specific resistance has recently been documented for three of the nine cultivars resistant to Races 3, 8, and 9, because infected plants have been identified in landscapes (Brite Eyes™ and Home Run®, unpublished data; Knock Out®, Whitaker and Hokanson, 2009b). Finding resistance-breaking pathogen isolates on cultivars characterized as resistant to all previously known races is a valuable tool in the identification of new races. Inoculating the remaining six of the nine rose cultivars with additional characterized races from the international collection (Whitaker et al., 2010b) and looking for infected plants in landscapes will help to identify infective isolates and confirm the presence of race-specific resistance.

Using laboratory assays to gain an understanding of partial resistance of cultivars may be the most immediate and predictive tool for identifying roses that have greater potential for durable field resistance to black spot. Finding similar rank order positions for LL for cultivars susceptible to multiple races is overall very promising. Whitaker et al. (2007b) found that among susceptible cultivars only, the *D. rosae* isolate $\times$ cultivar interaction was not significant for LL but was significant for other measures of partial resistance, including leaf area with symptoms and incubation period. In this study, however, the cultivar $\times$ race interaction for LL among cultivars susceptible to all races was significant (Table 5). This suggests that relative partial resistance across cultivars may change to some extent depending on which race (and possibly which isolate within a race) is en-

countered. Finding a positive correlation ($r = 0.618$; $P = 0.01$) between overall mean LL across races and the 2-year mean defoliation rating of Earth-Kind winning roses for the south-central United States is very promising. Comparing the rank of these 16 roses for LL and defoliation (Knock Out® was not included because it was not infected in this study and LL is not available), most cultivars were within only a few positions in rank across data types. The two most divergent roses were 'Caldwell Pink' and 'Ducher', which shifted in rank seven positions; ranks of 2 and 8 in field defoliation to 9 and 15 for LL, respectively. As long as cultivars do not change rank widely, characterization of a set of cultivars for partial resistance should still be informative, even as the races encountered in the field or additional laboratory inoculations change.

Many of the roses that have earned Earth-Kind designations in the south-central United States were susceptible to all three races (15 of the 17 roses) and displayed LLs that were consistently among the largest found (i.e., 'Belinda's Dream' and 'Climbing Pinkie'). In the field, such cultivars routinely become infected with black spot but consistently continue to flower well and retained much of their foliage throughout the growing season. It has been noted that some cultivars defoliated under lower disease pressure than others, an observation that has been confirmed by showing that LL is not necessarily related to frequency of defoliation (Whitaker et al., 2007b). A deficiency of the detached leaf inoculation method is its inability to measure the defoliation response, which can be influenced by rose cultivar response to ethylene and other factors (Whitaker and Hokanson, 2009b).

'Chorale' and 'Pariser Charme' were selected as controls for this study based on susceptibility to all three races and not for producing exceptionally large lesions after infection. 'Chorale' had consistently smaller LLs than 'Pariser Charme', and 'Pariser Charme' had the largest LLs of all in this study for the two races for which it was included as a control (Races 3 and 9) (Table 4). Future work could identify additional control cultivars with known susceptibility that can also serve as benchmarks for partial resistance levels. These control cultivars could be used both in laboratory inoculations and in the field.

'Belinda's Dream' has earned Earth-Kind designation in the south-central United States and is generally considered at the threshold of minimum black spot field resistance acceptable for a rose to earn Earth-Kind designation. More work can be done to correlate LL and a growing body of field black spot defoliation data to determine a minimum level of partial resistance based on laboratory assays for roses with Earth-Kind potential. Laboratory assays would be a very helpful tool to objectively eliminate those roses that clearly do not possess the minimum black spot resistance necessary to earn Earth-Kind designation to better allocate limited trialing resources. Besides possessing

Table 5. Mean squares from analysis of variance for effects resulting from cultivar, race, time of inoculation, and their interactions on lesion length calculated using only roses susceptible to *Diplocarpon rosae* Races 3, 8, and 9.

Factor	Mean square
Cultivar	4.64**
Race	4.90**
Time	7.19**
Cultivar $\times$ race	0.65*
Cultivar $\times$ time	1.20**
Race $\times$ time	0.70
Cultivar $\times$ race $\times$ time	0.40
Error	0.44

**Significant at $P \leq 0.01$; * significant at $P \leq 0.05$.

Table 6. Ploidy levels represented within the different commercial classes of roses challenged in this study (not including controls).

Class	No. Diploid	No. Triploid	No. Tetraploid
China	3		
Climbing floribunda			1
Climbing polyantha	1		
Floribunda		1	1
Hybrid kordesii		1	3
Hybrid musk	1	1	
Large-flowered climber		1	3
Polyantha	5		
Shrub	7	26	15
Tea	3		
Total cultivars	20	30	23

a minimum level of black spot resistance, a rose also needs to grow and flower reliably throughout the trialing region. Finding a significant, negative correlation ($r = -0.642$; $P < 0.01$) between our laboratory-based LL data and overall landscape performance ratings in a 3-year evaluation of the roses that eventually earned south-central U.S. Earth-Kind designation highlights the strong impact of black spot resistance on overall landscape performance of roses (Mackay et al., 2008).

Another approach to developing durably resistant cultivars would be for breeders to continue to identify race-specific black spot resistance genes and pyramid them into single cultivars (Whitaker and Hokanson, 2009b). To date, three race-specific resistance genes have been identified (Whitaker et al., 2010a). To more effectively pursue this approach, it will be necessary to identify more resistance genes and develop affordable molecular markers for marker-assisted selection. Although a cultivar may possess multiple race-specific resistances, the threat still exists for *D. rosae* isolates to emerge that possess the necessary combination of virulence alleles to overcome the resistance. The race-specific resistances described here for cultivars to Races 3, 8, and 9 provide a valuable starting point from which to begin to characterize additional race-specific resistance genes.

Blushing Knock Out® is a flower color sport of Knock Out®, and finding a difference in race-specific resistance to Race 8 between the two opens up research opportunities to characterize the genetic differences between these two closely allied cultivars (Table 1; Fig. 1). Blushing Knock Out® routinely sports back to Knock Out® and we isolated such a flower color reversion. We challenged the plant of Blushing Knock Out® from which the reversion was isolated and the Knock Out® reversion to Race 8 and confirmed susceptibility of Blushing Knock Out® and discovered that the reversion was re-

sistant to Race 8, just like the original Knock Out® (unpublished data). Perhaps the change leading to the color sport in Blushing Knock Out® is also associated with a change in resistance to Race 8. One possibility to explore is if Blushing Knock Out® is a Layer I periclinal chimera of Knock Out®, which can explain the common reversion to Knock Out® by meristem layer rearrangement. The epidermal layer in this case (derived from Layer I) may be housing some changes that have led to a change in anthocyanin pigmentation for the lighter color in petal epidermal cells and leaf surface changes leading to Race 8 susceptibility.

With black spot resistance being a high priority in the Explorer™ rose breeding program (Svejda, 2008), finding all of the roses evaluated in the series susceptible to Race 3 is of note. Uniform susceptibility to Race 3 across cultivars along with the prioritization of black spot resistance during the selection process together suggest that an isolate containing the virulence allele(s) present in Race 3 may not have been present at the trial locations during the selection of those cultivars.

A predominance of triploid roses was found in this study (Table 1). It has generally been accepted that most commonly sold modern commercial classes of roses (typically hybrid teas and floribundas) are tetraploid (Krüssmann, 1981). A high frequency of triploidy among popular landscape roses often sold as shrub roses has recently been reported by Zlesak (2009), and this study includes additional cultivars and helps to confirm this trend in landscape roses. Although some breeders may consciously be selecting for triploidy, it is more likely that this is an unintentional outcome of phenotypic selection. Triploidy can be associated with traits favorable in landscape roses, and triploidy has been documented to arise from any cross combination among diploid, triploid, and tetraploid parents (Zlesak, 2009).

It is known that ploidy level can affect plant morphology and fertility levels (Kermani et al., 2003; Leus, 2005; Rowley, 1960; Shahare and Shastry, 1963; Zlesak et al., 2005). In addition, changes in ploidy have recently been associated with changes in race-specific resistance between an induced tetraploid rose and the original diploid (Allum et al., 2010). Triploidy is typically associated with reduced fertility (Leus, 2005; Rowley, 1960), which can aid in reduced fruit set, freeing metabolic resources for faster regrowth and rebloom. Triploidy appears to offer a nice balance between traits typically associated with the diploid level (faster growth rates and greater branching) and tetraploid level (thicker, larger plant parts) (Zlesak, 2009; Zlesak et al., 2005). Documenting the ploidy levels of roses characterized for black spot resistance in this study can aid breeders in parental selection. Parents can be chosen not only for their resistance, but also fertility and anticipated ploidy level of their offspring.

Finding significant correlations of LL with field black spot ratings as well as overall performance ratings in south-central U.S. Earth-Kind rose field trials supports the use of such screens for objectively eliminating roses that do not meet a minimum level of resistance for inclusion in future Earth-Kind rose field trials. This large cultivar screen of diverse germplasm for resistance to *D. rosae* races and ploidy also opens doors for future research, including the identification of additional race-specific resistance genes, identifying new races of *D. rosae*, gaining insight to race composition in field trials based on cultivar infection patterns, selection of parents for resistance breeding efforts, and continued comparisons of laboratory LL data and a growing body of field resistance data.

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Fig. 1. Representative leaflets displaying response to Races 3 and 8 of *Diplocarpon rosae* for three roses within the Knock Out® series of cultivars.

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