Differential Response to Floral Infection by *Botrytis cinerea* within the Genus *Pelargonium*

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Abstract. The objective of this study was to identify geranium cultivars that exhibit differential reactions to floral inoculation with *Botrytis cinerea* Per. ex. Fr. Sixty-two genotypes, including both cultivars and breeding lines, were evaluated from several *Pelargonium* species. Resistant genotypes included the diploid *Pelargonium peltatum* (L.) L'Herit. cultivar King of Balcon and the diploid *Pelargonium ×hortorum* L.H. Bail. cultivar Ben Franklin, as well as the diploid *Pelargonium peltatum* accession 93-1-33 developed from an accession obtained from South Africa. Susceptible genotypes included the putative tetraploid *Pelargonium peltatum* cultivar Simone. Floral resistance was not correlated with foliar resistance. Diploid genotypes appeared to have greater resistance than tetraploid genotypes, and *P. peltatum* cultivars more resistance than *P. ×hortorum* cultivars. In addition, the association of petal number and resistance was investigated.

Botrytis blight or gray mold, caused by the fungus *Botrytis cinerea*, is one of the most common diseases of the zonal geranium. Flowers, foliage, stems, cuttings, and stock plants are all susceptible to attack by this pathogen. Hausbeck (1990) estimated that, in 1985, losses of geranium due to *B. cinerea* infection were \$U.S. 0.7 to 1.1 million in Pennsylvania and between \$5.1 and 7.6 million in the United States.

Flowers of many crops have been studied in an attempt to elucidate the mechanism(s) of resistance to *Botrytis* (Hammer and Evensen, 1994; Pie and De Leeuw, 1991; Salinas and Verhoeff, 1995; Sirjusingh and Sutton, 1996). Investigations into the *Pelargonium*–*Botrytis* pathosystem have been ongoing for many years. The initial description of the disease's epidemiology was provided by L.E. Melchers in 1918. Melchers noted that petals were the first tissue infected, and, as they abscised and dropped on the leaves, served as a nutrient

base for further fungal infection. Free water is required for infection by *Botrytis* (Salinas and Verhoeff, 1995), and at 21 °C a minimum wetness duration of 4 h is required for infection in *Pelargonium* (Sirjusingh and Sutton, 1996). Pie and De Leeuw (1991) observed direct penetration of rose (*Rosa hybrida* L.) petal cuticle through the formation of an infection peg, followed by the rapid degradation of the epidermal cell walls once the cuticle was penetrated. Salinas and Verhoeff (1995) observed direct penetration of *Gerbera* (*Gerbera ×hybrida* Hort.) petal cells, but the fungus did not form specialized structures and the cuticle did not appear to be degraded. Additionally, the presence of ethylene may play a role in disease establishment, as both rose and carnation (*Dianthus caryophyllus* L.), grown as cut flowers, were more susceptible to *B. cinerea* in the presence of ethylene (Elad, 1988).

Our approach initially was not to characterize the mechanism(s) in *Pelargonium*, but to evaluate the range of genetic floral resistance to *B. cinerea*. Cultivars with floral resistance may reduce the incidence of foliar infection by reducing the overall spore load within the greenhouse. While floral resistance to *B. cinerea* has been studied in many horticultural crops, few studies have evaluated multiple genotypes. There is some evidence of genetic variation for floral resistance to *Botrytis* among rose cultivars (Hammer and Evensen, 1994). Our objective was to compare 62 *Pelargonium* genotypes for genetic differences in floral resistance to *Botrytis*. We also investigated the association between resistance and ploidy levels, date of cultivar introduction, and species.

Materials and Methods

Plant culture. Sixty-two *Pelargonium* genotypes were grown under natural irradiance in a greenhouse at the Pennsylvania State

Univ. in 15-cm standard pots using Premier Pro-Mix BX (Premier Horticulture, Red Hill, Pa.) media. Plants were irrigated as needed and fertilized at each irrigation with Peter's Excel 15–5–15 (Scott Sierra, Marysville, Ohio) at a rate of 200 mg·L⁻¹ N. Temperature set points were 21 °C day/17 °C night, although these limits were often exceeded. Insect and arthropod pests were controlled using standard practices.

Pathogen culture. An isolate (M1) of *Botrytis cinerea* was collected as a single spore isolate from *Botrytis*-infected *Pelargonium* in a research greenhouse at the Pennsylvania State Univ. Conidia from this culture were put in long-term storage at 4 °C and the same isolate was used in all experiments. Cultures were initiated on a low-nutrient media composed of 20 g dextrose, 1 g K₂HPO₄, 0.5 g MgSO₄·7H₂O, 0.5 g KCl, 0.01 g FeSO₄·7H₂O, 1.7 g asparagine, and 20 g agar per liter of distilled water (Phillips et al., 1987). A 4-mm-diameter agar disk containing mycelium was used to inoculate leaves of the cultivar Ben Franklin. Leaves were incubated for 7 d at 21 °C under high humidity and 14-h photoperiod with irradiance provided by cool-white fluorescent tubes. After 7 d, conidia were washed from the leaf surface with autoclaved distilled water and used for inoculations.

Inoculations. Inflorescences were harvested from greenhouse-grown geraniums for floral inoculations. Individual florets were removed from the inflorescence at anthesis and placed in racks in plastic crisper boxes containing enough water to keep the base of the pedicel submerged. Florets were spray-inoculated with a *Botrytis* solution containing 4000 conidia/mL, using a Preval spray unit (Precision Valve Corp., Yonkers, N.Y.) until droplets of water appeared on the petals. Plants were incubated in a growth chamber for 2 d at 21 °C under the same conditions used for incubating leaves. Florets were evaluated visually on the following scale: 1 = no symptoms; 2 = small lesions on a few petals, barely visible to the naked eye; 3 = several small lesions, no lesions on at least one petal; 4 = lesions on all petals; 5 = many lesions, a few >2 mm; 6 = many lesions, several >2 mm; 7 = some lesions >4 mm, and/or an entire petal blighted; 8 = more than one petal entirely blighted; 9 = at least four petals entirely blighted. A cultivar with resistant flowers (but susceptible foliage), 'Ben Franklin' (Braun, 1992), and noninoculated florets were included in each trial. At least five florets were evaluated for each accession in each replication and all experiments were repeated three times.

In a second method of inoculation, 10 petals were removed from geranium florets and placed on moist filter paper in a petri dish. Each petal was inoculated with a 10-mL droplet of conidial suspension (4000 conidia/mL). Evaluations were made after 48 h, and the total number of diseased petals were counted.

Cell measurements. Petals were removed from florets of eight cultivars and the size of individual epidermal petal cells was measured using an ocular micrometer and a compound

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microscope. The cells of 10 petals were measured from three florets for each of the eight genotypes.

Statistical analysis. Data from four experiments were combined into a single table, since differences among experiments within the control genotype ('Ben Franklin') were non-significant in a one-way analysis of variance. Data were standardized prior to analysis by dividing floral ratings by the standard deviation of the population of each experiment.

Orthogonal contrasts were performed utilizing the standardized data to make meaningful comparisons among the accessions listed in Table 1. All floral evaluations were conducted as a randomized complete-block design. Data were analyzed using the GLM procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.).

Results and Discussion

A wide range of resistance was observed among genotypes, but none was immune. 'Ben Franklin' (diploid, *P. ×hortorum*), and 'King of Balcon' and accession 93-1-33 (both diploid, *P. peltatum*) were among the highly resistant genotypes (Table 1), and the ivy geranium (*P. peltatum*) cultivar Simone was by far the most susceptible genotype evaluated.

Genotypes with a standardized resistance value of <2 should be considered highly resistant (Table 1), those with a value >3 are relatively susceptible, and genotypes with a standardized value >4 should be considered highly susceptible. This information should prove useful to those who conduct research on floral resistance to *Botrytis* within the genus *Pelargonium*, and to those who wish to choose relatively resistant cultivars for breeding or production purposes.

Results using two inoculation methods (floret inoculation vs. individual petals) were not strongly correlated ($r = 0.51$). Only the floret evaluation was used for all experiments because it reasonably simulated natural inoculation methods and was the easiest method to use when evaluating many samples. Our inoculum level (4000 conidia/mL) was relatively high. Sirjusingh et al. (1996) reported that 10 conidia/mL was sufficient for infection of geranium tissue (Sirjusingh et al., 1996), and Braun (1992) suggested that 2000 conidia/mL was appropriate. However, we observed slightly more consistent results with the higher level (data not shown).

'Ben Franklin' was used as a resistant control, although it was among the most susceptible genotypes identified in our evaluations for foliar resistance (Uchneat et al., 1999). In fact, there seems to be no correlation between foliar and floral resistance, and each type of resistance needs to be considered a separate genetic trait. This should come as no surprise, as the physiology of leaf and floral tissues differ. In fact, resistance has been observed to be dependent on tissue and developmental stage in other crops (Ma and Singh, 1996; Makowski, 1993).

As *Botrytis* conidia germinate, the germ tube grows along the junctures of cells prior to

Table 1. Floral evaluations for resistance to *Botrytis* of greenhouse-grown pelargoniums ranked by standardized values (Std X) from four experiments. Genotypes with the greatest floral resistance are listed at the top of the table. Actual values (X) in each experiment are provided along with the standard error (SE of X) of the mean for these values.

Genotype	Species	Ploidy	Year ²	Std X	X ³	SE of X
King of Balcon	<i>P. peltatum</i>	2x	<1990 ⁴	1.10	1.70	0.21
93-1-33	<i>P. peltatum</i>	2x	1993	1.34	2.46	0.31
Ben Franklin	<i>P. ×hortorum</i>	2x	1987	1.41	2.58	0.32
Marilyn	floribunda	2x	1990	1.45	2.87	0.42
93-6-11	<i>P. ×hortorum</i>	2x	1993	1.57	2.89	0.42
Princess Balcon	<i>P. peltatum</i>	2x	<1990	1.59	2.92	0.38
86-23-1	<i>P. peltatum</i>	2x	1986	1.62	2.50	0.38
93-1-29	<i>P. peltatum</i>	2x	1993	1.63	3.00	0.55
93-6-7	<i>P. ×hortorum</i>	2x	1993	1.83	3.36	0.66
<i>P. acreum</i>	<i>P. acreum</i>	2x	---	1.86	3.00	0.53
Ritz	<i>P. ×hortorum</i>	4x	1992	1.95	3.87	0.70
93-1-4	<i>P. peltatum</i>	2x	1993	1.98	3.63	0.50
Minicascade Red	<i>P. peltatum</i>	2x	<1990	2.01	3.10	0.47
Minicascade Pink	<i>P. peltatum</i>	2x	<1990	2.02	3.71	0.50
<i>P. tongaense</i>	<i>P. tongaense</i>	2x	---	2.09	3.38	0.57
Minicascade Lavender	<i>P. peltatum</i>	2x	<1990	2.26	3.50	0.81
<i>P. zonale</i>	<i>P. zonale</i>	2x	---	2.26	3.65	0.51
93-5-4	<i>P. ×hortorum</i>	2x	1993	2.27	3.67	0.54
93-2-2	<i>P. ×hortorum</i>	2x	1993	2.30	3.72	0.45
Elizabeth	floribunda	2x	1993	2.45	4.86	0.61
93-3-1	<i>P. ×hortorum</i>	2x	1993	2.48	4.00	0.48
93-5-2	<i>P. ×hortorum</i>	2x	1993	2.52	4.06	0.66
84-72-5	<i>P. ×hortorum</i>	4x	1984	2.54	3.92	0.57
93-6-10	<i>P. ×hortorum</i>	2x	1993	2.54	4.67	0.66
93-2-3	<i>P. ×hortorum</i>	2x	1993	2.55	4.11	0.57
93-5-1	<i>P. ×hortorum</i>	2x	1993	2.58	4.17	0.54
Double Lilac White	<i>P. peltatum</i>	4x	<1971	2.59	4.75	0.59
Nicole	<i>P. peltatum</i>	4x	1990	2.60	4.78	0.74
Sunbelt Coral	<i>P. ×hortorum</i>	4x	1986	2.60	5.15	0.72
93-5-10	<i>P. ×hortorum</i>	2x	1993	2.66	4.89	0.50
Tetra Scarlet	<i>P. ×hortorum</i>	4x	>1990	2.70	4.17	0.71
93-6-6	<i>P. ×hortorum</i>	2x	1993	2.85	5.23	0.66
Mrs. Parker	<i>P. ×hortorum</i>	2x	1893	2.86	5.25	0.57
Sunset	<i>P. ×hortorum</i>	4x	1991	2.86	5.67	0.65
Fox	<i>P. ×hortorum</i>	4x	1989	2.91	4.50	0.32
Salmon Queen	<i>P. peltatum</i>	4x	<1990	2.92	5.78	0.74
Grace	floribunda	2x	1990	3.03	6.00	0.58
Melody	<i>P. ×hortorum</i>	4x	1992	3.07	6.07	0.60
Fame	<i>P. ×hortorum</i>	4x	---	3.09	4.77	0.50
Danielle	<i>P. ×hortorum</i>	4x	1987	3.13	6.20	0.50
93-3-5	<i>P. ×hortorum</i>	2x	1993	3.14	5.06	0.55
93-5-3	<i>P. ×hortorum</i>	2x	1993	3.14	5.06	0.70
Julia	floribunda	2x	1995	3.21	5.90	0.62
Precious	<i>P. ×hortorum</i>	4x	1992	3.30	6.53	0.52
Yours Truly	<i>P. ×hortorum</i>	4x	<1972	3.34	5.17	0.57
Medallion Dark Red	<i>P. ×hortorum</i>	4x	1993	3.38	5.22	0.62
Juliet	<i>P. ×hortorum</i>	4x	1989	3.38	6.70	0.83
93-5-5	<i>P. ×hortorum</i>	2x	1993	3.45	5.56	0.47
Judy	floribunda	2x	1990	3.47	6.87	0.57
<i>P. multibracteatum</i>	<i>P. multibracteatum</i>	2x	---	3.54	5.71	0.69
Angela	floribunda	2x	1995	3.56	5.50	0.50
Sassy Dark Red	<i>P. ×hortorum</i>	4x	1992	3.56	5.50	0.47
Veronica	<i>P. ×hortorum</i>	4x	1983	3.56	5.50	0.49
93-3-2	<i>P. ×hortorum</i>	2x	1993	3.61	5.83	0.44
White Nicole	<i>P. peltatum</i>	4x	1990	3.76	6.90	0.59
Sincerity	<i>P. ×hortorum</i>	4x	<1968	3.84	5.94	0.37
93-3-9	<i>P. ×hortorum</i>	2x	1993	3.89	6.28	0.57
93-3-7	<i>P. ×hortorum</i>	2x	1993	4.01	6.47	0.64
Snowwhite	<i>P. ×hortorum</i>	4x	1984	4.06	6.28	0.60
<i>P. inquinans</i>	<i>P. inquinans</i>	2x	---	4.06	6.56	0.53
Pink Expectations	<i>P. ×hortorum</i>	4x	1984	4.10	6.33	0.49
Simone	<i>P. peltatum</i>	4x	1990	5.25	8.11	0.26
Mean				2.82		
LSD _{0.05}				0.71		

²Year patented (if available) or introduced, data were obtained from Craig (1993) and Linda Wiles (personal communication) of Oglevee Ltd.

³1 = no symptoms; 2 = small lesions on a few petals, barely visible to the naked eye; 3 = several small lesions, no lesions on at least one petal; 4 = lesions on all petals; 5 = many lesions, a few greater than 2 mm; 6 = many lesions, several greater than 2 mm; 7 = some lesions greater than 4 mm, and/or an entire petal blighted; 8 = more than one petal entirely blighted; 9 = at least four petals entirely blighted.

⁴"<" indicates a release prior to this year, but exact date unknown.

Table 2. Comparison of *Pelargonium* cultivars categorized into various classes and compared for relative floral *Botrytis* resistance by using orthogonal contrasts and standardized *Botrytis* resistance ratings.

Comparison and mean values ^z	P value
Diploid cultivars (2.34) vs. tetraploid cultivars (3.18)	<0.00
<i>P. ×hortorum</i> (3.11) vs. <i>P. peltatum</i> (2.61)	0.05
Floribundas (2.86) vs. <i>P. peltatum</i> (2.61)	0.98
<i>P. ×hortorum</i> (3.11) vs. floribundas (2.86)	0.67
Cultivars since 1990 (3.18) vs. older cultivars (2.74)	0.40
Single-flowered (2.43) vs. double-flowered genotypes (2.98) ^y	0.10
Single-flowered (3.07) vs. double-flowered genotypes (2.57) ^x	0.01

^zNumbers in parentheses are mean standardized resistance ratings for that category.^yAll genotypes.^xOnly crosses involving 'Marilyn' and 'Ben Franklin'.

infection (Kamoen, 1992). We have observed this type of growth in *Pelargonium* tissue, but not exclusively (data not shown). Measurements were made on cell size (data not shown) with the expectation that smaller cells might have more cell junctures, which would be favorable to *Botrytis* germings. However, the correlation between cell size and relative floral resistance to *Botrytis* was nonsignificant ($r = 0.57$, $P = 0.14$) among eight genotypes. This coefficient increased to $r = 0.78$ ($P = 0.04$) when the cultivar Marilyn was removed from the analysis. In the latter case, smaller-celled cultivars had slightly better *Botrytis* resistance.

Diploid cultivars appeared to have greater resistance than tetraploid genotypes across all species and flower types (Table 2). Both single- (5 petals) and double- (>5 petals) flowered genotypes existed in each class (diploid and tetraploid). *Pelargonium peltatum* genotypes had greater resistance than *P. ×hortorum* genotypes, but the ivy geranium group was composed of primarily single-flowered genotypes, including some that were Penn State floral-resistant breeding lines. Single-flowered genotypes may have increased resistance, since the microclimate may be less humid in areas where only five petals exist compared to 10 or more petals. However, examination of progeny of crosses between 'Ben Franklin' and 'Marilyn' showed that double-flowered genotypes had greater resistance than did single-flowered ones with similar genetic backgrounds, although the sample size was small (11 progeny).

Pelargonium inquinans (L.) L'Herit. and *Pelargonium zonale* (L.) L'Herit. are generally considered to be the two most important

progenitors of *P. ×hortorum* (Harvey and Saunder, 1894; Horn, 1993). *Pelargonium zonale* was significantly more resistant to *Botrytis* than was *P. inquinans*. Several genotypes of *P. zonale* should be evaluated for *Botrytis* resistance, as these genotypes are easily cross-pollinated with most diploid cultivars and could provide greater resistance. This contrasts with mite resistance, where *P. inquinans* contributes resistance (Grazzini, 1993; Schultz, 1996).

Significant variation in *Botrytis* resistance was observed among the genotypes, suggesting that cultivars with greater *Botrytis* resistance can be developed. Data presented provide a scientific basis for selecting future parents. The identification of control genotypes for evaluating *Botrytis* resistance will be immensely valuable for future experiments.

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