Differential Response to Foliar Infection with Botrytis cinerea within the Genus Pelargonium

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ABSTRACT. Foliar evaluations for Botrytis resistance of greenhouse grown plants were performed on 45 cultivars and control genotypes including diploid and tetraploid zonal (P. ×hortorum L.H. Bailey) and ivy (P. peltatum L.) pelargoniums. Additional evaluations were performed on eight species within section Ciconium and on progeny of a susceptible by susceptible cross-pollination involving the cultivars Ben Franklin and Marilyn. Differential levels of resistance were observed. Among many genotypes that exhibited resistance, two genotypes had consistently high levels of Botrytis resistance over several experiments. These two genotypes were the diploid P. peltatum accession 86-23-1 and the tetraploid P. ×hortorum cultivar Fox. The diploid P. ×hortorum cultivar Ben Franklin was a reliable susceptible control in all experiments. Plants grown outdoors generally had higher levels of resistance than comparable greenhouse grown plants. Orthogonal contrasts indicated no trends in resistance when comparing diploid and tetraploid pelargoniums, or when comparing among ivy, zonal, and floribunda types. Genotypes patented or introduced since 1990 have greater susceptibility than older genotypes. Cross-pollinations among susceptible parents resulted in susceptible progeny, while self-pollinations of a resistant parent resulted in resistant progeny.

Geraniums (Pelargonium ×hortorum L.H. Bailey) are a major floricultural crop in the United States with a wholesale value exceeding $186 million dollars in 1994 (USDA, 1995). Botrytis blight or gray mold, caused by the fungus Botrytis cinerea Pers.:Fr., 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 due to Botrytis infection were 0.7 to 1.1 million dollars in Pennsylvania and between 5.1 and 7.6 million dollars in the United States. In addition, significant losses in plant quality may further reduce the plants’ value to the grower and the consumer.

Recent investigations concern the role of conidia as inoculum for geranium foliar and floral tissue. Sirjusingh and Sutton (1996) demonstrated that at least 4 h of wetness at 21 °C is required for foliar infection and sporulation of B. cinerea on geranium. Longer periods of wetness were needed for less ideal temperatures. Leaves <1 week old or >10 weeks old were more susceptible than 4-week-old leaves. Sirjusingh et al. (1996) also observed greater incidence of infection when infected petals were used to inoculate leaves compared to spores. Braun (1992) determined that less statistical variation was observed when colonized agar was used as inoculum compared to colonized petal tissue, probably due to the better standardization of the inoculum potential of the pathogen.

There are several methods of controlling Botrytis in geranium. Chemicals are frequently used, but populations of the fungus developed resistance to benimidazole and dicarboximide fungicides (Katan et al., 1989; Moorman and Lease, 1992). Environmental modifications may be effective (Hausbeck et al., 1996a, 1996b), and biological control may be possible as those technologies are improved (Sutton and Peng, 1993; Sutton et al., 1997). Additionally, genetic resistance through biotechnological methods may be possible, as well as the development of resistant geraniums through traditional plant breeding methods. Each of these methods has advantages and disadvantages, and success in controlling botrytis blight may depend on using several or all of these strategies in an integrated manner.

This paper discusses the investigation of foliar resistance to botrytis blight. A simple foliar assay was developed and used to survey available germplasm for relative resistance. Relative levels of resistance were compared among several species of Pelargonium in section Ciconium, among progeny of controlled cross-pollinations, between diploid and tetraploid genotypes, and between older and more recently introduced cultivars. This information should be valuable to plant breeders for utilization in the development of future cultivars with increased Botrytis resistance, as well as to current propagators and producers who will now have evidence of relative resistance in available cultivars.

Methods and Materials

PLANT CULTURE. Plants were grown in a research greenhouse at Pennsylvania State Univ. in 15-cm standard pots using Premier Pro-Mix BX (Premier Horticulture Inc., Red Hill, Pa.) media under natural irradiance. Plants were irrigated as needed and fertilized at

Table 1. Summary of germplasm evaluated for Botrytis resistance.

| Genetic group | Genotypes (no.) |
|---------------|-----------------|
| Tetraploid P. ×hortorum cultivars | 18 |
| PSU breeding lines1 | 7 |
| Floribunda pelargoniums (diploid) | 6 |
| Diploid P. peltatum cultivars | 5 |
| Tetraploid P. peltatum cultivars | 4 |
| Diploid P. ×hortorum cultivars | 4 |
| Other | 1 |
| Total | 45 |

1PSU breeding lines do not include cultivars that have been released by Pennsylvania State Univ.
each irrigation with Peter’s Excel 15–5–15 (Scotts Sierra, Marysville, Ohio) at a rate of 200 ppm N. Temperature set points were 21 °C during the day and 17 °C at night, though these temperatures were often exceeded. Insects and arthropod pests were controlled using standard practices.

**Pathogen Culture.** An isolate (M1) of *B.cinerea* was collected from *Botrytis* infected *Pelargonium* in a greenhouse at Pennsylvania State Univ. Infected leaves were collected and a single spore isolate was selected. Conidia from this culture were put in long term storage at 4 °C so that the same isolate was used in all experiments. For inoculations, cultures were initiated on a low-nutrient agar (LNA) medium composed of 20 g dextrose, 1 g

Table 2. Species, ploidy level, year patented or introduced, and relative resistance of 45 *Pelargonium* genotypes grown under greenhouse conditions, which were evaluated for foliar resistance using the detached leaf assay. Genotypes are ranked by relative resistance determined from standardized values (Std. X). True mean lesion diameter in mm (X) and standard error estimates are provided as they were calculated in individual experiments.

| Genotype              | Species             | Ploidy | Year | Std. X | X    | SE of X |
|-----------------------|---------------------|--------|------|--------|------|---------|
| 86-23-1               | *P. peltatum*       | 2x     | 1986 | 0.99   | 9.2  | 1.7     |
| 86-23-1C              | *P. peltatum*       | 2x     | 1996 | 1.23   | 11.4 | 2.3     |
| Fox                   | *P. xhortorum*      | 4x     | 1989 | 1.63   | 20.0 | 2.1     |
| 96-9-1 (86-23-1 x Steu1973) | *P. peltatum* | 2x     | 1996 | 1.76   | 16.3 | 2.6     |
| Red Elite             | *P. xhortorum*      | 2x     | <1990 | 1.77   | 16.6 | 2.1     |
| Steu 1973*            | *P. peltatum*       | 2x     | <1990 | 2.03   | 27.6 | 5.6     |
| Princess Balcon       | *P. peltatum*       | 2x     | <1990 | 2.12   | 28.8 | 4.4     |
| Minicascade Red       | *P. peltatum*       | 2x     | <1990 | 2.15   | 25.9 | 3.5     |
| Snowwhite             | *P. xhortorum*      | 4x     | 1984 | 2.32   | 28.0 | 3.0     |
| Aurora                | *P. xhortorum*      | 4x     | <1983 | 2.33   | 21.9 | 2.1     |
| Sassy Dark Red        | *P. xhortorum*      | 4x     | 1992 | 2.36   | 28.5 | 4.4     |
| King of Balcon        | *P. peltatum*       | 2x     | <1990 | 2.41   | 29.1 | 2.7     |
| Sunbelt Coral         | *P. xhortorum*      | 4x     | 1986 | 2.46   | 26.3 | 3.4     |
| Yours Truly           | *P. xhortorum*      | 4x     | <1972 | 2.47   | 29.8 | 4.3     |
| Laura                 | *P. xhortorum*      | 4x     | 1989 | 2.49   | 26.6 | 2.7     |
| Angela                | floribunda          | 2x     | 1995 | 2.55   | 30.7 | 3.4     |
| Minicascade Lavender  | *P. peltatum*       | 2x     | <1990 | 2.60   | 31.4 | 4.1     |
| White Nicole          | *P. peltatum*       | 4x     | 1990 | 2.69   | 24.9 | 3.0     |
| Nanette               | *P. peltatum*       | 4x     | 1990 | 2.73   | 25.6 | 3.8     |
| Pink Expectations     | *P. xhortorum*      | 4x     | 1984 | 2.74   | 33.1 | 4.0     |
| 71-17-7               | *P. xhortorum*      | 2x     | 1971 | 2.75   | 25.8 | 2.5     |
| Sincerity             | *P. xhortorum*      | 4x     | <1968 | 2.76   | 33.3 | 4.0     |
| Ritz                  | *P. xhortorum*      | 4x     | 1992 | 2.83   | 30.3 | 2.0     |
| 94-2-1                | Marilyn x 86-23-1    | 2x     | 1994 | 2.84   | 26.7 | 2.3     |
| Double Lilac White    | *P. peltatum*       | 4x     | <1971 | 2.86   | 38.9 | 3.4     |
| Nicole                | *P. peltatum*       | 4x     | 1990 | 2.86   | 38.9 | 7.2     |
| Grace                 | floribunda          | 2x     | 1990 | 2.93   | 31.3 | 3.6     |
| Juliet                | *P. xhortorum*      | 4x     | 1989 | 2.94   | 31.4 | 3.4     |
| Stadlbern             | *P. xhortorum*      | 2x     | <1978 | 2.99   | 28.1 | 2.5     |
| Julia                 | floribunda          | 2x     | 1995 | 3.00   | 40.7 | 3.0     |
| Medallion Dark Red    | *P. xhortorum*      | 4x     | 1993 | 3.02   | 32.3 | 3.4     |
| Precious              | *P. xhortorum*      | 4x     | 1992 | 3.02   | 32.3 | 3.1     |
| Sunset                | *P. xhortorum*      | 4x     | 1991 | 3.08   | 33.0 | 1.3     |
| Minicascade Pink      | *P. peltatum*       | 2x     | <1990 | 3.14   | 37.9 | 3.7     |
| Mrs. Parker           | *P. xhortorum*      | 2x     | 1893 | 3.17   | 43.0 | 3.8     |
| Elizabeth             | floribunda          | 2x     | 1993 | 3.18   | 34.0 | 3.5     |
| Ben Franklin (nonvar.) | *P. xhortorum*     | 2x     | 1987 | 3.23   | 43.9 | 3.4     |
| Marilyn               | floribunda          | 2x     | 1990 | 3.27   | 30.3 | 2.4     |
| Judy                  | floribunda          | 2x     | 1990 | 3.28   | 35.1 | 2.1     |
| Veronica              | *P. xhortorum*      | 4x     | 1983 | 3.32   | 35.5 | 3.4     |
| Danielle              | *P. xhortorum*      | 4x     | 1987 | 3.46   | 37.0 | 2.5     |
| Jubilee               | *P. xhortorum*      | 4x     | 1993 | 3.53   | 32.7 | 2.0     |
| Ben Franklin          | *P. xhortorum*      | 2x     | 1987 | 3.64   | 34.2 | 2.7     |
| Simone                | *P. peltatum*       | ?      | 1990 | 3.69   | 44.5 | 2.9     |
| Melody                | *P. xhortorum*      | 4x     | 1992 | 3.95   | 42.3 | 2.6     |

Data were obtained from Craig (1993) and Linda Wiles (personal communication) of Oglevee Ltd.

Data from seven individual experiments were standardized by dividing lesion diameter values after 5 d by the standard deviation of the population in each experiment and combined to create this table.

The designation <1990 indicates a release before 1990, but the exact date was not obtained.

Accessions beginning with the letters Steu were obtained courtesy J.J.A. van der Walt of the Univ. of Stellenbosch, South Africa.
RESULTS AND DISCUSSION

A sample of many currently available Pelargonium genotypes was evaluated in these experiments (Tables 1 and 2). After repeated testing, we discovered two genotypes that are consistently more resistant than other Pelargonium genotypes. The genotypes, 'Fox' (tetraploid, P. ×hortorum) and accession 86-23-1 (diploid, P. peltatum) consistently have a lesion diameter of ≈20 mm after 5 d, whereas other genotypes have lesion diameters that average 25 to 45 mm after 5 d. 'Fox' is an asexually propagated cultivar, which was bred in Dresden, Germany, and patented in 1989. Accession 86-23-1, first identified as resistant by Braun (1992), is a wild accession collected from South Africa. It behaves as an inbred and little variation is observed among self-pollinated progeny.

'Ben Franklin', a diploid cultivar, can be reliably used as a single table, since there were no significant differences when two control genotypes (Ben Franklin and accession 86-23-1) were compared across experiments in a one-way analysis of variance. Data were standardized before analysis by dividing lesion diameter values by the standard deviation of the population. Resistance rankings were reported as relative resistance based on standardized lesion diameters so that data from several experiments could be combined and reported in a single table. Evaluations of cultivars and readily available breeding lines are included in Table 2. Results from other experiments including evaluations of species within section Ciconium, and progeny of controlled cross-pollinations are reported in separate tables.

Each foliar evaluation was conducted as a randomized complete block experimental design. Data were analyzed using the GLM procedure of the Statistical Analysis System (SAS Institute, Cary, N.C.).

Orthogonal contrasts were used to make meaningful comparisons among the accessions listed in Table 2. Some genotypes were included in multiple experiments, though only the median value appears in Table 2. In cases where a genotype was included in several experiments, the median value was used in the orthogonal contrasts. In cases where only two dissimilar values were obtained, that genotype was excluded from this analysis. In cases where there were two similar values, the value from the experiment with the higher coefficient of determination was utilized for further analysis.

Table 3. Comparison of control genotypes to several Pelargonium species within section Ciconium. Control genotypes include accession 86-23-1 (resistant) and 'Ben Franklin' (susceptible).

| Genotype     | Species             | Ploidy | Mean lesion diam of (mm) | SE X |
|--------------|---------------------|--------|-------------------------|------|
| Fox          | P. ×hortorum        | 4x     | 8.9                     | 1.5  |
| Jubilee      | P. ×hortorum        | 4x     | 18.0                    | 2.5  |
| 86-23-1      | P. peltatum         | 2x     | 20.9                    | 1.5  |
| Steu 754^   | P. frutetorum       | 2x     | 23.5                    | 2.9  |
| Steu 2902    | P. multibracteatum  | 2x     | 29.3                    | 4.5  |
| Steu 2198    | P. cayae            | 4x     | 29.5                    | 3.5  |
| Steu 779     | P. transvaalense    | 2x     | 33.3                    | 3.7  |
| Steu 4271    | P. alchemilloides s.l. | 2x | 35.7                    | 4.0  |
| Ben Franklin | P. ×hortorum        | 2x     | 37.2                    | 1.7  |
| Steu 4270    | P. elongatum        | 2x     | 41.9                    | 1.6  |
| Mean         |                     |        | 27.8                    |      |
| LSD0.05      |                     |        | 7.4                     |      |

^Means are presented for lesion diameter 5 d after inoculation with a 4-mm-diameter agar disk colonized by Botrytis.

Accessions beginning with the letters Steu were obtained courtesy J.J.A. van der Walt of the Univ. of Stellenbosch, South Africa.
Table 4. Comparison of control and parental genotypes to several *Pelargonium* progeny involving cross pollinations between ‘Ben Franklin’ (BF) and ‘Marilyn’ (MAR). Each genotype is an individual accession descended from the described cross-fertilization.

| Genotype  | Species      | Pedigree        | Ploidy | Mean lesion diam (mm) | SE X |
|-----------|--------------|-----------------|--------|-----------------------|------|
| 86-23-1   | *P. peltatum* | ---             | 2x     | 12.3                  | 2.2  |
| 93-5-1    |              | MAR ⊗           | 2x     | 26.7                  | 3.0  |
| 93-3-1    |              | BF × MAR        | 2x     | 27.9                  | 3.7  |
| 93-2-3    |              | MAR × BF        | 2x     | 27.3                  | 2.7  |
| 93-3-5    |              | BF × MAR        | 2x     | 30.1                  | 3.4  |
| Marilyn   | floribunda   | ---             | 2x     | 30.3                  | 2.4  |
| 93-6-6    |              | MAR × BF        | 2x     | 31.3                  | 4.4  |
| 93-5-5    |              | MAR ⊗           | 2x     | 32.5                  | 3.5  |
| 93-6-4    |              | MAR × BF        | 2x     | 33.9                  | 2.6  |
| 93-3-9    |              | BF × MAR        | 2x     | 35.0                  | 2.5  |
| 93-3-8    |              | BF × MAR        | 2x     | 35.1                  | 3.5  |
| 93-5-3    |              | MAR ⊗           | 2x     | 36.3                  | 2.8  |
| 93-5-2    |              | MAR ⊗           | 2x     | 36.5                  | 2.5  |
| 93-3-7    |              | BF × MAR        | 2x     | 37.1                  | 3.3  |
| 93-6-11   |              | MAR × BF        | 2x     | 37.1                  | 3.1  |
| 93-3-2    |              | BF × MAR        | 2x     | 38.1                  | 2.5  |
| Ben Franklin | *P. × hortorum* | ---             | 2x     | 39.6                  | 4.5  |
| 93-6-7    |              | MAR × BF        | 2x     | 41.4                  | 4.1  |
| Mean      |              |                 |        | 32.9                  |      |
| LSD0.05   |              |                 |        | 7.9                   |      |

3Means are presented for lesion diameter 5 d after inoculation with a 4-mm-diameter agar disk colonized by *Botrytis*.

3These genotypes are listed as *P. × hortorum*, though they are partially comprised of other species, notably *P. peltatum* and *P. frutetorum*.

3Indicates a self-fertilization.

Susceptible control. This genotype has lesion diameters in excess of 40 mm after 5 d. This genotype is variegated, but an all green leaved mutant is as susceptible as the variegated form, suggesting that the variegation does not play a significant role in *Botrytis* susceptibility in this cultivar.

Other cultivars of interest include a colchic tetraploid accession of 86-23-1, referred to as 86-23-1C, the diploid F₁ hybrid ‘Red Elite’, and accession Steu 1973 (diploid, *P. peltatum*). The colchic tetraploid version of accession 86-23-1 had levels of resistance comparable to that of accession 86-23-1, and may be more useful in breeding programs since almost all asexually and a few seed-propagated cultivars are tetraploid. ‘Red Elite’ is a currently available diploid geranium and it exhibits high levels of foliar resistance in our experiments. Steu 1973 is a wild diploid *P. peltatum* accession collected from South Africa. It does not self-pollinate as readily as accession 86-23-1, and its level of homozygosity is unclear. Accession 96-9-1 is a hybrid between accession 86-23-1 and Steu 1973 and it also shows a high level of foliar *Botrytis* resistance (Table 2).

Six genotypes within section Ciconium were evaluated in addition to genotypes of *P. × hortorum* and *P. peltatum* (Table 3). It is important to know the relative resistance of these genotypes as they are potentially cross-compatible with commercial geraniums (Horn, 1993). Variation in resistance exists among these species (Table 3), but no species had greater resistance than accession 86-23-1 or more susceptibility than ‘Ben Franklin’. The usefulness of these species should not be dismissed, however, as *P. frutetorum* had resistance near that of accession 86-23-1, and this species can be found in the background of the floribunda group of geraniums (Holden, 1990). Additionally, we only evaluated one accession within each of these species. It is possible that greater resistance will be discovered as more genotypes are evaluated within each of these species. Accession 86-23-1 was cross-pollinated with the diploid cultivar Marilyn of the floribunda group (Table 4). The floribunda group is composed of hybridizations among *P. peltatum*, *P. × hortorum* and *P. frutetorum* genotypes. The hybrids were pollen sterile and also had

Table 5. Lesion diameter 5 d after inoculation for six *Pelargonium* genotypes grown in the greenhouse and outdoors in a trial garden. Lesion diameter is presented in mean lesion diameter in mm measured at the widest point.

| Genotype | Overall mean | SE of X | Indoor mean | SE of X | Outdoor mean | SE of X |
|----------|--------------|---------|-------------|---------|--------------|---------|
| 86-23-1  | 17.4         | 1.9     | 20.7        | 2.9     | 14.2         | 2.4     |
| Fox      | 29.4         | 2.5     | 33.6        | 3.4     | 25.3         | 3.5     |
| Jubilee  | 31.6         | 2.3     | 43.9        | 1.9     | 19.3         | 2.6     |
| Juliet   | 33.8         | 2.5     | 42.8        | 3.0     | 24.8         | 3.1     |
| Marilyn  | 37.0         | 2.3     | 47.8        | 2.7     | 28.4         | 2.6     |
| Ben Franklin | 43.6       | 2.4     | 53.5        | 1.9     | 33.3         | 3.5     |
strongly reduced ovule fertility. ‘Marilyn’ is highly susceptible when evaluated in our foliar Botrytis resistance assay. The progeny of this cross (94-2-1), which exhibited characteristics of ‘Marilyn’ and accession 86-23-1, had an intermediate level of foliar Botrytis resistance, suggesting a genetic component to resistance.

Progeny from self-pollinations of ‘Marilyn’ and cross-pollinations of ‘Marilyn’ and ‘Ben Franklin’, both susceptible, were evaluated (Table 4). In all cases, progeny resulting from these pollinations were as susceptible as their parents, though variation in susceptibility was observed. Cross-pollinations between accession 86-23-1 (resistant) and ‘Ben Franklin’ (susceptible) were attempted, but no progeny resulted.

With detached leaf assays, rank of resistance values did not change for accession 86-23-1, and the cultivars Marilyn and Ben Franklin, when plants were grown indoors versus outdoors (Table 5). Rank of resistance changed slightly for other cultivars, but not due to significant differences in resistance. Outdoor-grown plants had significantly (p < 0.001) smaller lesions (mean lesion diameter 24 mm) compared to plants grown indoors (mean lesion diameter 40 mm). It is likely that outdoor plants were more resistant because they were hardened by the somewhat harsher growing environment. In apples it has been reported that wound healing significantly strengthens cell walls and results in areas that are resistant to infection by B. cinerea (Lakshminarayana et al., 1987). A thick cell wall would be one of the obstacles Botrytis must overcome to initiate infection in Pelargonium leaves (Elad and Evensen, 1995).

Analysis using preplanned orthogonal contrasts allowed further comparisons to be made (Table 6). Among the genotypes listed in Table 2, no significantly different levels of resistance were found between diploid and tetraploid genotypes, or when zonal, ivy, or floribunda types were compared. Analysis did confirm that cultivars patented or introduced since 1990 had significantly less foliar Botrytis resistance as a group than cultivars developed before 1990. This suggests current breeding programs are not emphasizing disease resistance when selecting for new cultivars.

Historically, there has only been one set of reports indicating Botrytis resistance in Pelargonium. Griffith Buck (1973a, 1973b, 1978a, 1978b, 1978c) released several tetraploid cultivars with enhanced resistance to botrytis blight based on field and greenhouse observations, though it appears that these genotypes were not involved in replicated evaluations for Botrytis resistance. In this paper we describe genotypes that have resistance that is reliably based on replicated pathological evaluations. The highest level of resistance we have detected in these experiments does not totally prevent disease; all genotypes evaluated are susceptible to botrytis blight to some degree. In our experiments, many currently grown genotypes were highly susceptible under our test conditions.

In all of our experiments it is likely that we are evaluating leaves when they are at their greatest level of resistance. Sirjusingh et al. (1996) indicate foliar susceptibility was high in 1-week-old leaves, decreased in 1- to 4-week-old leaves, and increased again in older leaves. Since we are harvesting leaves from the third through fifth node, it is likely that leaves we evaluate are between 1 and 4 weeks old. In fact, our own observations have indicated that leaves from the top one-third of the plant exhibit greater resistance compared to leaves of the bottom one-third of the plant (unpublished data), possibly due to phenomena related to senescence. Braun (1992) observed a significant correlation between lesion diameters of leaves of intact plants and those on detached leaves. In her experiments, lesion diameter was similar in both cases. This is strong evidence that our assay is a valid indicator of relative Botrytis resistance, and should assist growers in making decisions regarding Botrytis resistance.

Significant variation in Botrytis resistance was observed among the genotypes reported in this research, suggesting it may be possible to enhance Botrytis resistance within the genus Pelargonium through breeding. The identification of control genotypes for use in evaluating Botrytis resistance will be immensely valuable for future experiments. Data presented here provide a starting point for selecting parents in future breeding projects for improved foliar Botrytis resistance. The experiments reported here address the problem of foliar Botrytis resistance. It is important to remember that Botrytis can also attack flowers, stock plants and cuttings. Further research is necessary to address these concerns.

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