Resistance of Petunia Phenotypes to *Botrytis cinerea*

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Additional index words. *Petunia hybrida*, *P. axillaris*, *P. integrifolia*, disease resistance, gray mold, Botrytis blight, germplasm, artificial inoculation

Abstract. Although *Petunia hybrida* Vilm., a major bedding plant, is susceptible to many diseases, no formal disease resistance studies have been conducted. *Botrytis cinerea* Pers. ex Fr. is a ubiquitous pathogen, causing great damage to greenhouse-grown ornamental crops, including petunia. In this study, a screening procedure for *B. cinerea* resistance in petunia was developed and 48 diverse petunia phenotypes were screened for resistance to *B. cinerea* in two seasons, spring and fall. The range of variability for resistance to *B. cinerea* in petunia was wide and continuous. Spearman's rank correlation coefficients between seasons were significant and moderate. While the majority of phenotypes displayed less than a 10% difference in mean percent infection in spring vs. fall seasons, several phenotypes displayed large differences that require further testing. One cultivar, 'Pink Sensation Improved', exhibited low and consistent mean percent infection in both spring and fall and, therefore, may be a useful source of resistance to *B. cinerea* in petunia.

Botrytis diseases are the most common and among the most destructive diseases of greenhouse-grown ornamental crops (Agrios, 1988). Botrytis blight, or gray mold, is caused by *B. cinerea* Pers. ex Fr., a ubiquitous and non-host specific facultative parasite, which affects a very wide range of species, including petunia. Initial infection typically occurs on senescent tissue. Once established, *B. cinerea* may invade surrounding healthy tissue (Blakeman, 1980). Leaf spots caused by *B. cinerea* usually appear as sunken, pale brown lesions. Blossom blights and fruit rots caused by *B. cinerea* are characterized in later stages by the appearance of distinctive conidia and conidiophores giving the fungus its common name, gray mold. Development of Botrytis blight is favored by a warm, humid environment and lush vegetative growth (Moody, 1991). These conditions are commonly found in production greenhouses where crowding and high nitrogen fertilization contribute to create optimal conditions for disease development. A combination of cultural controls, including increased ventilation, wider plant spacing, and proper fungicidal sprays, can be effective in controlling Botrytis blight. However, ever-rising costs of energy and labor encourage growers to maximize production in available greenhouse space. Fungicidal control is both environmentally and economically expensive. Demonstrated resistance of *Botrytis* strains to several commonly employed fungicides (Moorman, 1988) plus increasingly restrictive licensing policies for fungicides make alternative methods of control desirable. Identification of genetic resistance to Botrytis blight would provide a low-impact alternative to chemical con-

trol. No qualitative resistance to *Botrytis cinerea* is known to occur in any species (Jarvis, 1977). While some resistance to Botrytis blight has been observed in petunia cultivars (Ewart, 1984; Weddle, 1976), no study has been conducted to develop screening techniques for the identification of possible sources of resistance to *B. cinerea* in petunia. In the present study, a procedure for screening petunias for resistance to *B. cinerea* was developed and 48 diverse petunia phenotypes were screened for resistance.

Materials and Methods

Botrytis inoculum. Petunia flowers and leaves showing symptoms of Botrytis blight were collected from several different phenotypes of mature greenhouse-grown plants in the late fall of 1992. Segments cut from the margins of lesions were surface-sterilized for 1 min in 10% NaOCl, rinsed in sterile distilled water, and transferred to petri dishes containing potato-dextrose agar (PDA). Resulting mycelia were transferred to petri dishes containing PDA and grown at ambient laboratory temperature (23 to 25 °C) under continuous fluorescent light to promote sporulation.

Plant inoculation. The experiment was conducted in a greenhouse using a completely randomized design during two seasons, early spring and late fall 1993. Treatment 1 (natural inoculum) consisted of wounding plus application of sterile deionized water and ≈0.02% (v/v) Tween 20 detergent. Treatment 2 (artificial inoculum) consisted of wounding plus application of a suspension containing 1×10^6 spores/mL sterile deionized water and ≈0.02% (v/v) Tween 20. With each treatment, 10 leaves per plant were wounded by burning <5% of the upper mid-blade surface with a heated laboratory spatula tip (≈8 mm wide). A 10-mL droplet with or without inoculum was applied to each wound. Plants were randomly placed in a dew chamber maintained at 100% relative

humidity in an unheated greenhouse with no supplemental lighting. After 8 d, incidence of infection was assessed. Development of sunken brown lesions extending beyond the periphery of the burn wound was defined as *B. cinerea* infection and the fungus was re-isolated from these lesions. Each phenotype was treated and assessed three times (three replications) per season. Infection was calculated as the mean percentage of leaves out of a total of 30 (10 leaves per plant $\times$ three plants per phenotype) that developed spreading lesions.

Assessment of resistance. Forty-eight phenotypes of petunia, including modern and older cultivars, inbred breeding lines, and accessions of the purported progenitor species of *P. hybrida*, were screened for resistance to *B. cinerea*. Seeds of the nine inbred breeding lines (MSP 1, MSP 2, MSP 4, MSP 5, MSP 6, MSP 89-34A, MSP 90-14A, MSP 90-17A, and MSP90-53A) were supplied by Dr. Lowell Ewart of Michigan State Univ. Modern cultivars were donated by: Goldsmith Seeds, Gilroy, Calif.; Harris Seeds, Rochester, N.Y.; Pan American Seed, West Chicago, Ill.; Royal Sluis Seeds, Salinas, Calif.; S & G Seeds, Downers Grove, Ill.; and Sakata Seed America, Salinas, Calif. Older cultivars and accessions of the purported progenitor species of *P. hybrida* Hort., *P. axillaris* Lam., and *P. integrifolia* Hook., were obtained from the U.S. Dept. of Agriculture, Seed Storage Laboratory, Fort Collins, Colo.

Statistical analysis. Mean percent infection was analyzed using Proc ANOVA with multiple comparisons within season determined using Duncan's multiple-range test (SAS Institute, 1988). Spearman's correlation coefficients were calculated to assess consistency of phenotype rank order over seasons.

Results and Discussion

The two inoculation methods were analyzed separately because of the extremely high F value ($F = 4446.65$, $P < 0.0001$) resulting from a combined analysis, and a very high F value for the phenotype $\times$ treatment interaction. The phenotype $\times$ season interaction was significant for both treatment analyses; therefore, the phenotype $\times$ season term was used as the error term in the analysis of variance for each treatment (Table 1). Mean percent infec-

Table 1. Analysis of variance for mean percent infection of 48 petunia phenotypes by *Botrytis cinerea*.

Source	df	MS
<i>Treatment 1: wounding, natural inoculum</i>		
Season	1	0.40***
Phenotype ^a	47	0.21***
Phenotype $\times$ season	47	0.03*
Error	192	0.02
<i>Treatment 2: wounding, with artificial inoculum</i>		
Season	1	0.06*
Phenotype ^a	47	0.14***
Phenotype $\times$ season	47	0.03***
Error	192	0.01

^aPhenotype $\times$ season is the error term for testing phenotype effect.

*,***Significant at $P = 0.05$ or 0.001 , respectively.

Received for publication 20 Aug. 1998. Accepted for publication 23 Dec. 1998. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

tion differed significantly among phenotypes for both treatments in each season. Although the phenotype $\times$ season interaction was significant for each treatment, the estimate of the variance component for phenotype (using natural inoculum) was 10 times as great as the variance component for phenotype $\times$ season interaction. For artificial inoculation, the estimate of the variance component for phenotype was >2.5 times as great as the variance component for the phenotype $\times$ season interaction. Thus, the effect of season on the phenotypic expression for *B. cinerea* infection with each treatment was small.

Spearman's correlation coefficients between spring and fall season were significant for each inoculum treatment ($r=0.77$ and 0.65 for natural and artificial inoculum, respec-

tively; $P=0.05$) indicating a moderate level of phenotype rank order consistency between spring and fall for each treatment. However, Spearman's correlation coefficient for natural and artificial inoculum over seasons was extremely low and nonsignificant, indicating a lack of phenotype rank order consistency between the two treatments over seasons.

The range of variability in the 48 petunia phenotypes for mean percentage of infection by *B. cinerea* was wide and continuous (Tables 2 and 3). Using natural inoculum, the range in spring was from 0 to 77% mean infection, with a mean over all phenotypes of 25% (Table 2). The range in fall for natural inoculum was from 0 to 60% mean infection, with a mean over all phenotypes of 13%. Since spores of *B. cinerea* are ubiquitous in production green-

houses, a certain level of infection incidence for natural inoculum was expected. The study with natural inoculum may approximate the natural potential for *Botrytis* disease development occurring in clean greenhouses early in the growing season. Early in the production season, when plants are small and, well spaced within and between flats or pots, air circulation is good, and humidity within the plant canopy is relatively low, there is very little senescent tissue and no established *Botrytis* disease. The levels of infection observed in the phenotypes treated with natural inoculum resulted from ambient levels of *B. cinerea* spores in the atmosphere.

For treatment 2 (artificial inoculum), the range was the same in each season, from 23% to 100% mean infection, with a mean over all

Table 2. Mean percent infection of 48 petunia phenotypes by *Botrytis cinerea* in two seasons using natural vs. artificial inoculation.

Phenotype ^a	Mean percent infection			
	Natural inoculation		Artificial inoculation	
	Spring	Fall	Spring	Fall
Satellite	0 a	3 ab ^y	93 g-i	90 g-j
Linda	0 a	0 a	83 e-i	83 e-j
Venus	0 a	3 ab	83 e-i	83 e-j
Neptune	3 ab	3 ab	87 f-i	90 g-j
<i>P. axillaris</i> NSL 88812	3 ab	0 a	73 c-f	97 ij
Inca	3 ab	3 ab	67 c-e	80 d-i
Snowbird	3 ab	10 a-d	57 bc	67 d-f
Cherry Tart	3 ab	3 ab	60 b-d	73 d-g
Crusader	3 ab	3 ab	93 g-i	93 h-j
Pink Cheeks	3 ab	0 a	100 i	100 j
Pink Desire	3 ab	0 a	83 e-i	83 e-j
Comanche	7 a-c	0 a	33 a	70 d-g
MSP 90-53A	7 a-c	3 ab	97 hi	97 ij
MSP 6	7 a-c	0 a	100 i	100 j
Pink Sensation Improved	7 a-c	7 a-c	23 a	23 a
Howard's Star Improved	7 a-c	0 a	50 b	70 d-g
<i>P. integrifolia</i> NSL 88813	7 a-c	0 a	93 g-i	87 f-j
Calypso	10 a-c	0 a	87 f-i	83 e-j
Extra Dwarf Giant	10 a-c	3 ab	100 i	93 h-j
Comet	10 a-c	0 a	100 i	40 ab
Sugar Daddy	13 a-d	7 a-c	97 hi	97 ij
Primetime Blue	13 a-d	13 a-d	90 f-i	83 e-j
Purple Wave	13 a-d	0 a	100 i	100 j
Matador	13 a-d	7 a-c	93 g-i	77 d-i
MSP 5	17 a-e	7 a-c	100 i	90 g-j
Merlin Pink	20 a-e	0 a	93 g-i	83 e-j
Chiffon	20 a-e	0 a	77 d-g	93 h-j
Minuet	20 a-e	13 a-d	97 hi	97 ij
Bingo	23 a-f	10 a-d	100 i	100 j
Celebrity Red	23 a-f	7 a-c	87 f-i	87 f-j
Fandango	27 a-g	7 a-c	83 e-i	83 e-j
White Magic	30 b-h	3 ab	83 e-i	100 j
MSP 89-34A	33 c-i	20 c-e	100 i	93 h-j
<i>P. integrifolia</i> NSL 98077	33 c-i	40 g-i	100 i	100 j
MSP 4	40 d-i	20 c-e	80 e-h	80 d-j
Rose and White Pearls	43 e-j	10 a-d	77 d-g	60 cd
Celebrity Raspberry Ice	43 e-j	10 a-d	80 e-h	77 d-i
MSP 90-17A	50 f-k	23 d-f	100 i	100 j
Hullahoop Rose	50 f-k	7 a-c	97 hi	87 f-j
MSP 90-14A	53 g-k	20 c-e	100 i	100 j
Maestro	53 g-k	43 hi	100 i	90 g-j
Orchid Cloud	53 g-k	27 e-g	83 e-i	63 c-f
Ultra Rose Star	57 h-k	17 b-e	73 c-f	47 bc
MSP 1	60 i-k	40 g-i	97 hi	97 hi
Crimson Titan	70 jk	53 ij	73 c-f	80 d-j
MSP 2	70 jk	60 j	83 e-i	77 d-i
Postillion Salmon	73 k	37 f-h	90 f-i	67 d-f
Highlight Blue	77 k	53 ij	93 g-i	60 cd

^aSee text for seed sources.

^yMean separation within columns by Duncan's multiple range test, $P=0.05$; $n=3$ for each phenotype.

Table 3. Mean percent infection with *Botrytis cinerea* for 48 petunia phenotypes with treatment 2 (wounding plus artificial inoculum) in two seasons.

Phenotype	Mean percent infection	
	Spring	Fall
Pink Sensation Improved	23 a	23 a ^a
Comanche	33 a	70 d-g
Howard's Star Improved	50 b	70 d-g
Snowbird	57 bc	67 d-f
Cherry Tart	60 b-d	73 d-g
Inca	67 c-e	80 d-j
<i>P. axillaris</i> NSL 88812	73 c-f	97 ij
Ultra Rose Star	73 c-f	47 bc
Crimson Titan	73 c-f	80 d-j
Rose and White Pearls	77 d-g	60 cd
Chiffon	77 d-g	93 h-j
Celebrity Raspberry Ice	80 e-h	77 d-i
MSP 4	80 e-h	80 d-j
Venus	83 e-i	83 e-j
Pink Desire	83 e-i	83 e-j
Linda	0.83 e-i	83 e-j
MSP 2	83 e-i	77 d-i
Orchid Cloud	83 e-i	63 c-e
Fandango	83 e-i	83 e-j
White Magic	83 e-i	100 j
Neptune	87 f-i	90 g-j
Calypso	87 f-i	83 e-j
Celebrity Red	87 f-i	87 f-j
Postillion Salmon	90 f-i	67 d-f
Primetime Blue	90 f-i	83 e-j
Matador	93 g-i	77 d-i
<i>P. integrifolia</i> NSL 88813	93 g-i	87 f-j
Highlight Blue	93 g-i	60 cd
Merlin Pink	93 g-i	83 e-j
Crusader	93 g-i	93 h-j
Satellite	93 g-i	90 g-j
MSP 1	97 hi	97 ij
Sugar Daddy	97 hi	97 ij
Minuet	97 hi	97 ij
Hullahoop Rose	97 hi	87 f-j
MSP 90-53A	97 hi	97 ij
Bingo	100 i	100 j
<i>P. integrifolia</i> NSL 98077	100 i	100 j
Comet	100 i	40 ab
MSP 6	100 i	100 j
Purple Wave	100 i	100 j
MSP 90-14A	100 i	100 j
MSP 90-17A	100 i	100 j
MSP 89-34A	100 i	93 h-j
Maestro	100 i	90 g-j
Pink Cheeks	100 i	100 j
MSP 5	100 i	90 g-j
Extra Dwarf Giant	100 i	93 h-j

^aMean separation by Duncan's multiple range test, $P=0.05$; $n=3$ for each phenotype.

phenotypes of 85% and 83% infection for spring and fall, respectively (Table 3). The study with artificial inoculum may approximate the natural potential for the development of *Botrytis* disease occurring in production greenhouses when the disease potential is high due to a combination of conditions, including poor air circulation, high humidity in the dense plant canopy, and large amounts of senescent tissue.

The continuous range of variability for mean percentage of infection with natural and artificial inoculum suggests that resistance to *B. cinerea* in petunia is quantitative. A majority of the phenotypes, including the nine inbred breeding lines and the three accessions of the purported progenitor species of *P. hybrida*, exhibited little or no resistance when artificially inoculated. In contrast, many phenotypes expressed high levels of resistance, while others were highly susceptible when inoculated naturally. A majority (70%) of phenotypes ex-

hibited less than a 10% difference in mean infection between seasons when artificial inoculum was used. Several phenotypes, including 'Comet', 'Highlight Blue', 'Postillion Salmon', 'Orchid Cloud', 'Ultra Rose Star', *P. axillaris* NSL88812, 'Howard's Star', and 'Comanche' differed greatly between seasons, warranting further investigation. 'Pink Sensation Improved', an older cultivar no longer commercially available, exhibited low mean percent infection (23%) in both spring and fall when artificial inoculum was used. 'Pink Sensation Improved' may prove to be an important source of resistance to *B. cinerea* for use in breeding *Botrytis*-resistant petunia cultivars.

Literature Cited

- Agrios, G.N. 1988. Plant diseases caused by fungi, p. 356–425. In: G.N. Agrios (ed.). Plant pathology. Academic Press, New York.
- Blakeman, J.P. 1980. Behaviour of conidia on aerial plant surfaces, p. 225–248. In: J.R. Coley-Smith, K. Verhoeff, and W.R. Jarvis (eds.). The biology of *Botrytis*. Academic Press, New York.
- Ewart, L. 1984. Plant breeding, p. 180–202. In: K.C. Sink (ed.). Petunia: Monographs on theoretical and applied genetics. Springer-Verlag, New York.
- Jarvis, W.R. 1977. *Botryotinia* and *Botrytis* species: Taxonomy, physiology and pathogenicity. Monograph 15, Research Branch, Canada Dept. Agr., Ottawa.
- Moody, E.H. 1991. Coping with Botrytis blight—A serious disease of greenhouse grown ornamental plants in 1991. Ornamental plant pathology update. Ga. Coop. Ext. Circ. 1:4–6.
- Moorman, G.W. 1988. 1988 Diseases. Coop. Reg. Project NCR-43. Annu. Rpt.
- SAS Institute, 1988. SAS/STAT user's guide, release 6.03 ed. SAS Inst., Cary, N.C.
- Weddle, C.L. 1976. Petunias, p. 252–270. In: J.W. Mastalerz (ed.). Bedding plants: A manual on the culture of bedding plants as a greenhouse crop. Penn. Flower Growers, University Park.