Evaluation of Lisianthus Cultivars for Resistance to *Botrytis cinerea*

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ABSTRACT

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Lisianthus (*Eustoma grandiflorum*) is a high-value cut flower. However, major yield losses often result from gray mold caused by *Botrytis cinerea*. Various techniques were used to evaluate 12 lisianthus cultivars for resistance *B. cinerea*. Disease evaluations from detached leaf, leaf disc, cut stem, and in vivo growth chamber stem (GC) assays were correlated with those from an in vivo greenhouse stem (GH) assay, in which commercial greenhouse production of lisianthus was simulated. In all assays, stems or leaves were wounded before inoculation with spores or mycelia of *B. cinerea*. There was a significant ($P \leq 0.03$) positive correlation between stem lesion length in the GH assay and disease incidence in the same assay ($R = 0.74$), stem lesion length from spore spray inoculation in the GC assay ($R = 0.62$), and percent necrosis from spore spray inoculation of detached leaves ($R = 0.71$). Correlations between stem lesion length in the GH assay and disease evaluations from spore drop and mycelial inoculation of detached leaves, leaf discs, and cut stems were not significant at $P = 0.05$. Considering only screening methods with significant correlations, 'Magic Champagne' was the most resistant cultivar (mean rank [mr] = 2 of 12). 'Echo White' and 'Echo Lavender' were the least resistant cultivars (mr = 11). The other cultivars were 'Magic White' (mr = 4); 'Avila Ivory', 'Balboa Yellow', 'Echo Pink', and 'Magic Rose' (mr = 5); 'Balboa Blue' (mr = 6); 'Avila Blue Rim' (mr = 8); and 'Avila Purple' and 'Catalina Purple' (mr = 9). The results from this study indicate that in vivo disease incidence, in vivo stem assays, and detached leaf assays, all initiated with wounding followed by spore spray inoculation, may be more reliable in evaluating lisianthus cultivars for resistance to *B. cinerea* than spore drop and mycelial inoculation of detached leaves, leaf discs, and cut stems. The results also indicate that lisianthus cultivars with moderate resistance to *B. cinerea* are commercially available. These cultivars have potential for use as germplasm in breeding lisianthus for resistance to the pathogen.

Additional keywords: cut stem assay, leaf disc assay

Lisianthus (*Eustoma grandiflorum*) is a high-value cut flower. In 2005, the total wholesale value of lisianthus in the United States for operations with sales worth \$100,000 or more was \$4.89 million, with California accounting for 89.4% of those sales (17). Lisianthus entered the cut-flower market in the early 1980s when Sakata Seed Company introduced F-1 hybrid seed (7). Therefore, lisianthus is relatively new in the cut-flower industry. Little research has been done on diseases. In greenhouse production, gray mold caused by *Botrytis cinerea* is one of the major diseases affecting lisianthus. Symptoms of gray mold on lisianthus include crown rot, damping off, stem cankers, and stem, leaf, and flower blights. Gray mold also can cause postharvest deterioration of flowers.

Differences in cultivar susceptibility to *B. cinerea* in cut flowers such as rose (*Rosa* spp.) (9) and food crops such as strawberry (*Fragaria* × *ananassa*) (1,3) have been demonstrated. However, research has not been done to evaluate lisianthus cultivars for resistance to *B. cinerea*. In a series of vase life tests at a flower auction, Vrind (18) observed *Botrytis* infection in 15, 25, and 30% of all tests with roses, gerbera (*Gerbera jamesonii*), and lisianthus, respectively. Vase life of flower bunches with *Botrytis* spp. infection was reduced by 3 days in roses and gerbera and 6 days in lisianthus. In all, 50% of lisianthus cultivars at the auction were infected by *Botrytis* spp. compared with 16 and 22% of rose and gerbera cultivars, respectively. Vrind's study (18) demonstrates that lisianthus may be more susceptible to *Botrytis* spp. than other cut flowers of comparable value. Because gray mold causes considerable yield losses in lisianthus production, there is a need to identify cultivars with resistance or tolerance to the disease. Such cultivars can be used as germplasm in breeding efforts and, if used by growers, can reduce yield losses caused by gray mold.

Various techniques have been used to screen cultivars of various crops for resistance to diseases. The cut-stem technique

has been used by several investigators to evaluate soybean (*Glycine max*) cultivars for resistance to stem rot caused by *Sclerotinia sclerotiorum* (2,12,14,20). Hazendonk et al. (9) tested rose cultivars for postharvest susceptibility to *B. cinerea* by spraying flowers with a spore suspension. The detached leaf assay has been widely used to screen cultivars for resistance to various pathogens, including *S. sclerotiorum* on soybean (11,12,20), *Ascochyta rabiei* on chickpea (*Cicer arietinum*) (4), *Venturia inaequalis* on apple (*Malus* × *domestica*) (22), and *Erysiphe pisi* on pea (*Pisum sativum*) (16). A detached leaf disk assay was used to screen cherry (*Prunus* spp.) cultivars for susceptibility to powdery mildew caused by *Podosphaera clandestina* (15) and leaf spot caused by *Blumeriella jaapii* (21). Because studies have not been conducted to evaluate lisianthus cultivars for resistance to *Botrytis cinerea*, effective techniques for resistance screening have not been developed. Therefore, there is a need to identify reliable cultivar screening techniques.

The objectives of this study were to (i) develop techniques for screening lisianthus cultivars for resistance to *B. cinerea* and (ii) evaluate lisianthus cultivars for resistance to *B. cinerea*.

MATERIALS AND METHODS

From 2003 to 2004, methods for screening lisianthus cultivars for resistance to *B. cinerea* were evaluated to determine which ones were most effective. The most effective methods were used to evaluate 12 cultivars in each year for resistance to *B. cinerea*.

Origin and maintenance of pathogen isolate. The isolate of *B. cinerea* used in all experiments was obtained from an infected lisianthus plant in a commercial greenhouse in California. The isolate was grown on potato dextrose agar (PDA) and stored as mycelium on 9-cm-diameter petri plates at 4°C. Mycelial plugs from storage were used to start cultures needed to conduct experiments.

Growth and maintenance of plants. In 2003 and 2004, plugs of 12 lisianthus cultivars (288 plugs per tray) were transplanted into 10-cm pots, one plant (2003) or two plants (2004) per pot. The cultivars were chosen based on sales of plugs at a California nursery. The potting medium consisted of a mixture of plaster sand (26%), bark (37%), and peat moss (37%) and contained phosphorus at 160 ppm. Plants were grown in the greenhouse without supplemental light. Temperature in the

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greenhouse ranged from 22°C (nighttime low) to 32°C (daytime high). Plants were fertilized once every 2 weeks with a nutrient solution containing nitrogen, potassium, calcium, magnesium, sulfur, iron, molybdenum, zinc, copper, and manganese at 68, 33, 25, 18, 25, 0.89, 0.002, 0.00011, 0.00004, and 0.0001 ppm, respectively.

Inoculum preparation. Spores were harvested from 3- to 4-week-old *B. cinerea* cultures grown on PDA in 9-cm-diameter petri plates and incubated at 22°C in 12 h of light and 12 h of dark. Sterile distilled water was added to each culture plate and spores were dislodged with a rubber policeman. The spore suspension then was filtered through four layers of cheese cloth. Spore concentration was determined with a hemacytometer. Mycelial cultures used to inoculate leaves and stems were grown on PDA at 22°C in 12 h of light and 12 h of dark.

Harvesting leaves or stems and surface sterilization of leaves. Leaves were harvested from the top one-third of plants at the budding to flowering growth stage. Stems were cut with scissors approximately 1 cm above the soil line at the same growth stage and defoliated. Immediately after harvest, leaves and stems were transported to the laboratory in clear plastic boxes and experiments were set up on the same day. Leaves were surface sterilized in a 1:1 solution of 0.525% NaOCl and 95% ethanol for 5 s (2003) or in 5% ethanol for 30 s (2004). They then were rinsed in sterile distilled water and blotted dry in a laminar flow hood.

Incubation of leaves, leaf discs, or stems. In the laboratory, inoculated leaves, leaf discs, or stems were placed in 31.3-by-23.5-by-9.5-cm clear plastic boxes which then were covered with lids prior to incubation in continuous darkness at 16 or 16 and 22°C (detached leaf assay, spore spray inoculation in 2004). Leaves or leaf discs inoculated with mycelia were enclosed in petri plates containing 15 ml of water agar (WA). These petri plates were covered and put in clear plastic boxes which then were covered with lids prior to incubation. Except for experiments in which leaves or leaf discs were enclosed in WA petri plates, the interior sides of the clear plastic boxes and lids were sprayed with distilled water to maintain close to 100% relative humidity.

Experimental design. In all experiments, cultivars were arranged in a randomized complete block design with 3 replications (laboratory and growth chamber experiments), 17 replications (plastic chamber experiment in 2004), or 4 replications (leaf disc assay and spore drop inoculation in 2004). Each experiment was repeated once except the cut-stem assay, which was conducted twice in 2003 and once in 2004.

Disease assessment. Disease assessment keys (10) were used as a guide to

estimate percent necrotic area on leaves. Percent necrotic tissue on stems was calculated by dividing the length of necrotic tissue by total stem length and multiplying by 100.

2003. Method 1, detached-leaf assay–spore drop inoculation. A 2-mm-diameter wound was created in the center of each leaf (adaxial surface up) by pressing the sharp end of the center rod of a cork borer set through both surfaces of the leaf. The leaves were placed on wet paper towels in clear plastic boxes and 50 µl of a *B. cinerea* spore suspension (3.8×10^5 spores/ml) was dropped with a micropipette on each wound immediately after wounding and again 3 days later. The leaves were covered with wet paper towels following inoculation. Percent necrotic area was estimated 8 days after inoculation.

Method 2, detached-leaf assay–mycelial disc inoculation. Leaves were wounded as described above. Each leaf was placed with its adaxial surface up onto the surface of 15 ml of WA contained in a 9-cm-diameter petri plate. An 8-mm-diameter mycelial plug from the edge of a 4-day-old *B. cinerea* culture was placed over the wound with mycelia in contact with the wound. The petri plates containing inoculated leaves were covered and placed in a clear plastic box. Percent necrotic area was estimated 3 days after inoculation.

Method 3, cut-stem assay–spore spray inoculation. Stems were trimmed to 21 cm in length from the flower end and placed on wet paper towels in clear plastic boxes (one replication per box). A spray bottle was used to spray the stems with a *B. cinerea* spore suspension (8.4×10^5 spores/ml, 8 ml per replication) immediately after wounding and again 3 days later at 12 ml per replication. Lesion length was measured 10 days after inoculation.

2004. Method 1, in vivo stem assay in a plastic chamber–spore spray inoculation. This experiment was designed to simulate commercial production of lisianthus. Twelve cultivars were grown in the greenhouse in 10-cm pots as described above. The top 3 to 5 cm was clipped off each plant with scissors at the budding to flowering growth stage to create wounds. The plants were placed in a transparent plastic chamber (length by width by height = 4.3 by 1.8 by 1.1 m) built over a greenhouse bench. Immediately after wounding, a CO₂-powered, ceramic nozzle cone-type TXVK-10 backpack sprayer set at 221 kPa was used to inoculate the foliage and stems with 500 ml of a *B. cinerea* spore suspension (4.6×10^5 spores/ml). To maintain conditions favorable for infection in the plastic chamber, plants were sprayed with the same backpack sprayer on the foliage and stems to runoff three times each day with a mist of tap water. Average temperature in the plastic chamber was 23°C and average relative humidity was 61%. Lighting regime was natural (no artificial light-

ing was used). Lesion length on stems was measured 35 days after inoculation.

Method 2, disease incidence in the plastic chamber in method 1. Disease incidence (percentage of infected plants) in the plastic chamber described in method 1 was determined 35 days after inoculation.

Method 3, detached-leaf assay–spore spray inoculation. Leaves were wounded as described in method 1, 2003. They then were placed on wet paper towels in clear plastic boxes (12 cultivars, three replications per box). A *B. cinerea* spore suspension (1.2×10^6 spores/ml, 10 ml/box) was sprayed over the leaves with a Chromist spray unit (Gelman Instrument Company, Ann Arbor, MI). Leaves were incubated at 16°C (one box) and 22°C (one box). Percent necrotic area was estimated 5 days after inoculation.

Method 4, detached-leaf assay–mycelial disc inoculation. Three wounds approximately 2 mm apart were created with an inoculating needle in a triangular pattern near the edge of each leaf approximately halfway between the tip and base of the leaf. The wounds went through both surfaces of the leaf. Each wounded leaf was placed on 15 ml of WA in a 9-cm-diameter petri plate and a 5-mm-diameter PDA plug from the edge of an actively growing culture of *B. cinerea* was placed over the three wounds with mycelia in contact with the wounds. The petri plates containing inoculated leaves were covered and put in a clear plastic box. Percent necrotic area was estimated 6 days after inoculation.

Method 5, in vivo stem assay in a growth chamber–spore spray inoculation. At the nine-leaf stage, plants were wounded on two opposite leaves (one wound per leaf, complete penetration of the leaf blade) in the upper one-third of the stem with an inoculating needle and transferred to a growth chamber with a misting system (2 min of mist every 6 h, 15.5 h of leaf wetness/day, 20°C average temperature, 12 h of light and 12 h of dark). Plants were inoculated with a spray bottle on the foliage and stems to runoff with a *B. cinerea* spore suspension (5×10^5 spores/ml, 95 ml). Lesion length on the stems was measured 17 days after inoculation.

Method 6, cut-stem assay–spore spray inoculation. Stems were trimmed at the flower end to 21 cm length, placed in clear plastic boxes (one replication per box), and covered with paper towels, leaving 0.5 cm at the lower end exposed. They were inoculated on the exposed lower ends with a *B. cinerea* spore suspension (1.2×10^6 spores/ml, 10 ml/replication) using a Chromist spray unit (Gelman Instrument Company). Percent necrotic length was determined 14 days after inoculation.

Method 7, leaf-disc assay–spore drop inoculation. A 13-mm-diameter tissue disc was cut from the center of each leaf with a cork borer and an inoculating needle was used to make three wounds at the center of

the disc. The wounds were approximately 2 mm apart, formed a triangular shape, and went through both surfaces of the tissue disc. Wounded tissue discs were placed on 30-by-2.54-cm white (noncolorized) wooden stakes. Twelve tissue discs representing 12 cultivars were placed linearly on each of four wooden stakes. The distance between tissue discs was approximately 1 cm. Each tissue disc was inoculated by dropping 20 µl of a *B. cinerea* spore suspension (1.2×10^6 spores/ml) over the three wounds with a micropipette. The four wooden stakes with inoculated leaf discs were placed on wet paper towels in a clear plastic box. Percent necrotic area was estimated 4 days after inoculation.

Method 8, leaf disc assay–mycelial culture inoculation. Tissue discs (13 mm in diameter) were cut out of leaves as in method 7, except they were not wounded in the center. They were placed in a circular pattern on the edges of 4-day-old *B. cinerea* cultures growing on PDA in 9-cm-

diameter petri plates. Twelve tissue discs representing 12 cultivars were placed on each of three petri plates. The petri plates were covered and placed in a clear plastic box. Percent necrotic area was estimated 4 days after inoculation.

Data analysis. Data from 2003 and 2004 experiments were analyzed separately because one cultivar in 2003 was replaced by a different cultivar in 2004. Data were analyzed by the general linear models procedure of SAS (SAS Institute, Cary, NC). A combined analysis of replicate experiments was done based on homogeneous error variances determined from Levene's test (13). In method 3 in 2004 (detached leaf assay–spore spray inoculation), cultivar–temperature interaction was not significant at $P = 0.05$ and error variance among the four experiments was homogeneous according to Levene's test (13). Therefore, a combined analysis of the four experiments was done. Because a large number of plants (68 plants per

cultivar in two experiments) was evaluated in the in vivo stem assay in method 1 in 2004, and because disease development in this method was similar to disease development in a commercial greenhouse, data from all other methods were correlated with data from method 1 in 2004. The correlation coefficients (R) from linear correlation analysis (data from each method correlated with data from method 1 in 2004) were used as a measure of accuracy of each method in detecting resistance or susceptibility of lisianthus cultivars to *B. cinerea*. In all experiments, Fisher's least significant difference test (6) at $P = 0.05$ was used to compare pairs of treatment means.

RESULTS

2003. In method 1 (detached leaf assay–spore drop inoculation), the F value for cultivar was not significant ($P = 0.8410$). Percent necrotic area on leaves was significantly ($P = 0.05$) lower in 'Avila Ivory' than in all other cultivars and ranged from 10% in Avila Ivory to 43% in 'Echo White' (Table 1). In method 2 (detached leaf assay–mycelial inoculation), percent necrotic area differed among cultivars ($P = 0.0026$) and ranged from 18% in 'Avila Blue Rim' to 42% in Echo White (Table 1). In method 3 (cut stem assay–spore spray inoculation), percent necrotic stem length differed among cultivars ($P = 0.0002$) and was lowest in 'Balboa Yellow' and Avila Ivory (48%) and highest in 'Echo Lavender' (93%) (Table 1).

2004. Methods 1 (in vivo stem assay in a plastic chamber), 2 (disease incidence in the plastic chamber in method 1), 3 (detached leaf assay–spore spray inoculation), 5 (in vivo stem assay in a growth chamber), and 7 (leaf disc assay–spore drop inoculation) detected differences in resistance to *B. cinerea* among the 12 lisianthus cultivars, whereas methods 4 (detached leaf assay–mycelial disc inoculation), 6

Table 1. Responses and ranks of 12 lisianthus cultivars evaluated for resistance to *Botrytis cinerea* with three inoculation and evaluation methods in 2003

Cultivar	Evaluation method ^z			Rank		
	1	2	3	1	2	3
	Necrotic (%)	Necrotic (%)	Lesion (%)			
Echo White	43 a	42 a	83 a	12	12	9
Echo Lavender	34 ab	20 d	93 a	7	2	12
Avila Purple	31 ab	39 ab	60 bc	5	11	7
Avila Blue Rim	36 ab	18 d	56 bc	10	1	5
Magic White	33 ab	23 cd	59 bc	6	4	6
Magic Rose	30 ab	26 cd	50 c	4	7	3
Balboa Purple	35 ab	36 a–c	55 bc	8	10	4
Balboa Yellow	23 ab	24 cd	48 c	2	5	1
Balboa Blue	35 ab	22 d	76 ab	9	3	8
Echo Pink	41 ab	25 cd	91 a	11	6	11
Avila Ivory	10 b	29 b–d	48 c	1	8	2
Magic Champagne	27 ab	30 b–d	84 a	3	9	10

^z Method 1, detached leaf assay–spore drop inoculation; method 2, detached leaf assay–mycelial disc inoculation; and method 3, cut stem assay–spore spray inoculation. Necrotic = necrotic leaf area and Lesion = stem lesion length. Cultivar means within a column followed by the same letter are not significantly different according to Fisher's least significant difference test at $P = 0.05$.

Table 2. Responses of 12 lisianthus cultivars evaluated for resistance to *Botrytis cinerea* using eight inoculation and evaluation methods in 2004

Cultivar	Evaluation method ^z							
	1	2	3	4	5	6	7	8
	Lesion (cm)	Disease (%)	Necrotic (%)	Necrotic (%)	Lesion (cm)	Lesion (%)	Necrotic (%)	Necrotic (%)
Echo White	15 a	79 ab	55 ab	26 a	10 a–c	48 b	43 a–c	61 a
Echo Lavender	13 ab	85 a	57 a	43 a	9 b–d	48 b	54 ab	54 a
Avila Purple	12 a–c	77 a–c	49 a–c	49 a	6 de	87 a	66 ab	65 a
Avila Blue Rim	12 a–d	69 a–d	35 b–e	49 a	13 a	49 b	71 a	69 a
Catalina Purple	10 b–e	69 a–d	41 a–d	36 a	13 ab	39 b	68 a	44 a
Magic White	9 c–e	68 a–d	20 ef	28 a	4 ef	46 b	35 bc	46 a
Magic Rose	9 c–e	65 b–d	24 e–f	31 a	8 c–e	46 b	46 a–c	53 a
Balboa Yellow	9 c–e	57 de	39 a–e	32 a	5 de	51 b	50 ab	63 a
Balboa Blue	9 de	59 cd	38 a–e	38 a	9 b–d	46 b	62 ab	68 a
Echo Pink	8 e	78 ab	14 f	30 a	5 de	36 b	15 c	56 a
Avila Ivory	7 ef	71 a–d	25 e–f	28 a	8 cd	39 b	52 ab	55 a
Magic Champagne	4 f	40 e	30 c–f	35 a	0 f	43 b	36 bc	70 a

^z Method 1, in vivo stem assay in a plastic chamber–spore spray inoculation; method 2, disease incidence in the plastic chamber in method 1; method 3, detached leaf assay–spore spray inoculation; method 4, detached leaf assay–mycelial disc inoculation; method 5, in vivo stem assay in a growth chamber–spore spray inoculation; method 6, cut stem assay–spore spray inoculation; method 7, leaf disc assay–spore drop inoculation; and method 8, leaf disc assay–mycelial culture inoculation. Lesion = stem lesion length, Disease = disease incidence, and Necrotic = necrotic leaf area. Cultivar means within a column followed by the same letter are not significantly different according to Fisher's least significant difference test at $P = 0.05$.

(cut stem assay–spore spray inoculation), and 8 (leaf disc assay–mycelial culture inoculation) did not (Table 2). Each of the five methods that detected differences among cultivars consistently identified ‘Magic Champagne’ as more resistant and Echo White and Echo Lavender as more susceptible to *B. cinerea* than other cultivars (Table 2). The rest of the cultivars were variable in their reaction to *B. cinerea* in the various evaluation methods (Table 2).

Cultivar ranks. In 2003, cultivar ranks varied widely among the three evaluation methods. Balboa Yellow and ‘Magic White’ ranked 6 or better (rank 1 = most resistant; rank 12 = least resistant) whereas Echo White ranked 9 or worse in all three methods (Table 1). In 2004, Magic Champagne and ‘Magic Rose’ ranked 6 or better in methods 1, 2, 3, 5, and 7, which detected differences among cultivars (Table 3). Echo Lavender ranked 8 or worse in all five methods, whereas Echo White ranked 8 or worse in four of the five methods which detected differences among cultivars (Table 3).

Determination of reliable screening methods. To determine which evaluation methods were reliable in screening for resistance to *Botrytis* spp., the in vivo stem assay in a plastic chamber (method 1), in which large numbers of each cultivar were screened, was used as the standard, and disease assessments in each of methods 1 to 3 in 2003 and 2 to 8 in 2004 were correlated with disease assessments in this method. Method 1 was considered the standard because it simulated disease development in commercial greenhouse production of lisianthus. In 2004, disease incidence (method 2, $R = 0.74$), spore spray inoculation of detached leaves (method 3, $R = 0.71$), and lesion lengths on stems of live plants spray inoculated with a spore suspension followed by incubation in a growth chamber with a misting system (method 5, $R = 0.62$) were significantly ($P \leq 0.03$) correlated with lesion lengths on live plants spray inoculated with a spore suspension and incubated in a plastic chamber on a greenhouse bench (method 1, standard; Table 4). Considering only

screening methods with significant correlations, Magic Champagne was the most resistant (mean rank [mr] = 2 of 12) and Echo White and Echo Lavender were the least resistant (mr = 11). Other cultivars and their ranks were Magic White (mr = 4); Avila Ivory, Balboa Yellow, ‘Echo Pink’, and Magic Rose (mr = 5); ‘Balboa Blue’ (mr = 6); Avila Blue Rim (mr = 8); and ‘Avila Purple’ and ‘Catalina Purple’ (mr = 9).

DISCUSSION

Accuracy of identifying disease-resistant cultivars is dependent on reliability of screening methods. Because this is the first study to screen lisianthus cultivars for resistance to *B. cinerea*, several screening techniques were tested and their reliability was measured by correlating disease assessments in these techniques to disease assessments in a standard assay in which conditions in commercial greenhouse production of lisianthus were simulated. With this approach, it was demonstrated that an in vivo stem assay, in vivo

Table 3. Ranks of 12 lisianthus cultivars (1 = most resistant; 12 = least resistant) evaluated for resistance to *Botrytis cinerea* using eight inoculation and evaluation methods in 2004

Cultivar	Rank per evaluation method ^z							
	1	2	3	4	5	6	7	8
Echo White	12	11	11	1	10	9	4	7
Echo Lavender	11	12	12	10	8	8	8	4
Avila Purple	10	9	10	11	5	12	10	9
Avila Blue Rim	9	6	6	11	12	10	12	11
Catalina Purple	8	6	9	8	11	2	11	1
Magic White	7	5	2	3	2	5	2	2
Magic Rose	5	4	3	5	6	6	5	3
Balboa Yellow	5	2	8	6	3	11	6	8
Balboa Blue	4	3	7	9	9	7	9	10
Echo Pink	3	10	1	4	4	1	1	6
Avila Ivory	2	8	4	2	7	2	7	5
Magic Champagne	1	1	5	7	1	4	3	12

^z Method 1, in vivo stem assay in a plastic chamber–spore spray inoculation, stem lesion length (cm) (length); method 2, disease incidence (%) in the plastic chamber in method 1; method 3, detached leaf assay–spore spray inoculation, necrotic leaf area (%) (necrotic); method 4, detached leaf assay–mycelial disc inoculation (necrotic); method 5, in vivo stem assay in a growth chamber–spore spray inoculation, (length); method 6, cut stem assay–spore spray inoculation, stem lesion length (%) (lesion); method 7, leaf disc assay–spore drop inoculation (necrotic); and method 8, leaf disc assay–mycelial culture inoculation (necrotic).

Table 4. Correlation between measurements in each method used to evaluate 12 lisianthus cultivars for resistance to *Botrytis cinerea* in 2003 and 2004 with lesion lengths on stems of live plants wounded at the top, inoculated with spores of *B. cinerea*, and incubated in a plastic chamber on a greenhouse bench in 2004

Year, assay–inoculation method	No. of experiments	N ^w	R ^x	Prob > R ^y
2003				
1. Detached leaf assay–spore drop inoculation	2	11	0.48	0.1369
2. Detached leaf assay–mycelial disc inoculation	2	11	0.24	0.4813
3. Cut stem assay–spore spray inoculation	2	11	0.05	0.8804
2004				
1. In vivo stem assay in a plastic chamber–spore spray inoculation	2	...	...	...
2. Disease incidence in the plastic chamber in method 1	2	12	0.74	0.0055
3. Detached leaf assay–spore spray inoculation	4	12	0.71	0.0092
4. Detached leaf assay–mycelial disc inoculation	2	12	0.33	0.2956
5. In vivo stem assay in a growth chamber–spore spray inoculation	2	12	0.62	0.0310
6. Cut stem assay–spore spray inoculation	1	12	0.44	0.1481
7. Leaf disc assay–spore drop inoculation	2	12	0.43	0.1580
8. Leaf disc assay–mycelial culture inoculation	2	12	–0.10	0.7641

^w Number of cultivars used in correlation analysis.

^x Pearson correlation coefficient.

^y Probability of a greater Pearson correlation coefficient.

^z Values are not given because all other methods were correlated with this method.

disease incidence, and a detached leaf assay, all initiated with spore spray inoculation, were more reliable than spore drop or mycelial inoculation of detached leaves, leaf discs, or cut stems.

Within the three screening methods found to be reliable, there were fluctuations in cultivar ranking. Previous studies have reported similar inconsistency in reaction of cultivars of various crops screened for resistance to pathogens. Wegulo et al. (20) found ranks of 12 soybean cultivars to fluctuate among several methods in which the cultivars were screened for resistance to *S. sclerotiorum*, causal agent of Sclerotinia stem rot of soybean. Daubeny and Pepin (3) screened strawberry cultivars for susceptibility to postharvest fruit rot and found most of the results to agree with field observations. However, they also observed some inconsistencies, which they ascribed to variation in the degree of maturity of test fruit. In the study reported here, stems and leaves but not flowers were used for screening in order to avoid variation among cultivars in time to flowering. The fluctuations observed in cultivar ranks among the different screening methods may be due in part to differences in the defense arsenal among lisianthus cultivars. The defense strategies of some cultivars may vary with the screening method, the environment, or the time of year. The cultivars evaluated in this study were grown under the same greenhouse conditions in late summer to fall in 2003 and 2004. However, some of the cultivars, such as those in the Echo series, grow best during early spring, whereas those in the Balboa and Catalina series are good for summer production in greenhouses.

Results from this study indicate that there are commercially available lisianthus cultivars with moderate resistance to *B. cinerea*. Based on the more comprehensive experiments of 2004 (compared with the three experiments of 2003), and considering only screening methods we found to be reliable (in vivo stem assay, in vivo disease incidence, and the detached leaf assay, all initiated with spore spray inoculation), cv. Magic Champagne was the most resistant, whereas Echo White and Echo Lavender were the least resistant. None of the cultivars included in the study was immune to *B. cinerea* or had a high level of resistance to the pathogen. Based on the data reported in this study, even Magic Champagne, which was the most resistant, can best be described as having moderate resistance to

B. cinerea. Vrind (18) observed that lisianthus cultivars generally were more susceptible to *B. cinerea* than rose and gerbera cultivars. In addition to traits such as flower color, stem length and strength, and vase life, it is imperative that lisianthus breeders consider disease resistance a priority in their breeding efforts.

Few studies have been done to evaluate lisianthus cultivars for resistance to diseases. In addition to the study of Vrind (18) mentioned above, Harbaugh and McGovern (8) evaluated 46 lisianthus cultivars for resistance to *Fusarium avenaceum*. They found that, although some plants of all cultivars were susceptible to the pathogen, some cultivars exhibited partial resistance based on differences in time from inoculation to symptom expression. *F. avenaceum* is one of the most serious pathogens of lisianthus (*personal observation* in commercial greenhouse production in California). Gilardi et al. (5) found 4 of 12 lisianthus cultivars to have some level of resistance to *F. oxysporum* f. sp. *eustomae* based on disease incidence. Wang and McSorley (19) evaluated cultivars in several genera of cut flowers for resistance to the root knot nematode, *Meloidogyne incognita*. They found lisianthus to be one of two genera that were poor hosts of the nematode. These studies demonstrate that there are lisianthus cultivars with some level of resistance to diseases. More studies are needed to screen lisianthus cultivars for resistance to diseases and lisianthus breeders are encouraged to place more emphasis on disease resistance.

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