Journal of Phytopathology

J Phytopathol **160**:88–97 (2012)
doi: 10.1111/j.1439-0434.2011.01862.x

Otis A. Floyd Nursery Research Center, Tennessee State University, McMinnville, TN, USA

Evaluation of *Hydrangea macrophylla* for Resistance to Leaf-Spot Diseases

Margaret T. Mmbaga[1], Mee-Sook Kim[2], Lucas Mackasmiel[1] and Yonghao Li[3]

Authors' addresses: [1]Tennessee State University, School of Agriculture and Consumer Science, Otis A. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110, USA; [2]Department of Forestry, Environment, and Systems, Kookmin University, Seoul 136-702, Korea; [3]Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, New Haven, CT 06511, USA (correspondence to M. T. Mmbaga. E-mail: mmmbaga@tnstate.edu)

Received April 25, 2011; accepted September 22, 2011

Keywords: *Corynespora cassiicola*, *Cercospora* spp., *Glomerella cingulata*, *Colletotrichum gloeosporioides*, *Phoma exigua*, *Myrothecium roridum*, *Botrytis cinerea*, *Hydrangea macrophylla*, resistance selections

Abstract

Garden hydrangea (*Hydrangea macrophylla*) is a popular ornamental plant that can be devastated by leaf-spot diseases. Information is needed to determine susceptibility of commercial cultivars to leaf-spot diseases. To address this need, 88 cultivars of *H. macrophylla* were evaluated for their resistance to leaf-spot diseases in full-shade (2007–2008), full-sun (2007–2008) and partial-shade (2009–2010) environments in McMinnville, TN, USA. Ten cultivars ['Ami Pasquier', 'Ayesha', 'Blue Bird', 'Forever Pink', 'Fuji Waterfall' ('Fujinotaki'), 'Miyama-yae-Murasaki', 'Seafoam', 'Taube', 'Tricolor' and 'Veitchii'] were rated resistant (R) or moderately resistant to leaf spot under each of the three environments. In 2007–2008, approximately 51% of the cultivars were rated R in full shade, but only 5% were R in full sun. In 2009–2010, only 1% of the cultivars were rated R in partial shade. Although environmental parameters including temperature and rainfall influence disease severity and host reaction, a shaded environment was least favourable for leaf-spot disease development, which demonstrates that establishing hydrangea in shaded environment can be an effective tool along with cultivar selection for managing leaf-spot diseases on hydrangea. Six pathogens, *Corynespora cassiicola*, *Cercospora* spp., *Myrothecium roridum*, *Glomerella cingulata* (Anamorph: *Colletotrichum gloeosporioides*), *Phoma exigua* and *Botrytis cinerea*, were associated with leaf-spot diseases of garden hydrangea. Of the leaf-spot pathogens, *C. cassiicola* was most frequently isolated (55% of all isolates), followed by *Cercospora* spp. (20%) and other pathogens (25%). Because symptoms attributed to each leaf-spot pathogen were similar, cultivars were selected for resistance to multiple leaf-spot pathogens.

Introduction

Garden hydrangea (*Hydrangea macrophylla*), which includes bigleaf hydrangea, French hydrangea and florist hydrangea, is a popular landscape shrub planted in full sun, partial shade or shade, and it is also grown as a potted plant. Flowers of some cultivars can be dried and used in floral arrangements and crafts. It is commonly believed that hydrangeas need only moisture and shade to thrive, but most cultivars benefit from dappled direct sunlight or exposure to morning sun (Vintage Gardens 2008). Hydrangeas are easy to grow and provide colour in gardens from mid-summer through autumn, and they can be used as specimen plants in shrub borders. Over 700 cultivars of *H. macrophylla* are described, but only about one-fourth are available in the USA (Dirr 2004; van Gelderen and van Gelderen 2004). Hydrangeas grow from 0.9 to 1.8 m in height with a similar spread and produce large flowers in early to mid-summer. While four subspecies of *H. macrophylla* have been recognized (McClintock 1957), only two subspecies, *H. macrophylla* ssp. *macrophylla* and *H. macrophylla* ssp. *serrata*, are grown commercially in the USA. The two subspecies can be differentiated by their leaf and inflorescence sizes (McClintock 1957), a combination of several qualitative and quantitative morphological traits (Bertrand 2000), or molecular markers (Reed and Rinehart 2007). *Hydrangea macrophylla* ssp. *macrophylla* is native to Japan and is found in coastal areas from sea level to approximately 150 m in elevation (Bailey 1989). *Hydrangea macrophylla* ssp. *serrata*, also known as mountain hydrangea, is found at higher elevations up to 1500 m and is cultivated primarily as a garden plant. Although several inconsistencies are associated with the cultivar names (Haworth-Booth 1984), a cultivar checklist is available in Bertrand (2001).

Leaf-spot diseases can have significant impact on hydrangea health, appearance and market value. In the landscape, leaf-spot diseases cause unsightly appearance of foliage. Severe defoliations are sporadic and have been associated with frequent rainfall. Repeated defoliations can reduce overall plant vigour (Vann 2010). In nursery production of hydrangea, leaf-spot diseases become problematic when plants are exposed to frequent rainfall or overhead irrigation (Williams-Woodward and Daughtrey 2001). Various fungal pathogens cause leaf-spot diseases in *H. macrophylla* (Sinclair et al. 1987; Hagan and Mullen 2001; Williams-Woodward and Daughtrey 2001). Fungal pathogens associated with hydrangea leaf spots include *Ascochyta*, *Botrytis*, *Cercospora*, *Colletotrichum*, *Corynespora*, *Phyllosticta* and *Septoria* species (Williams-Woodward and Daughtrey 2001). Cercospora leaf spot causes heavy damage on container-grown plants in nursery production, and once the disease becomes established in an area, disease outbreaks are likely to occur every year (Hagan and Mullen 2001). Hot weather and frequent rain showers favour disease outbreaks (Hagan and Mullen 2001; Hagan et al. 2004). Thus, the environment of south-eastern USA is highly favourable to hydrangea leaf-spot diseases, and the main limiting factor to leaf-spot diseases is the availability of inocula or susceptible hosts. The objectives of this study were to (i) evaluate numerous *H. macrophylla* cultivars for resistance to leaf-spot diseases and (ii) identify pathogens involved with leaf-spot diseases in *H. macrophylla*.

Materials and Methods

Plant materials

This study was conducted at Tennessee State University, Otis L. Floyd Nursery Research Center (TSU-NRC) in McMinnville, TN, USA. Two sets of rooted cuttings of 88 commercial cultivars of *H. macrophylla* were grown in 26.5-l, plastic containers. The study comprised 68 *H. macrophylla* ssp. *macrophylla* (*mac*), 17 *H. macrophylla* ssp. *serrata* (*serrata*) cultivars and three hybrids between the two subspecies (Table 1). Subspecies assignment for cultivars used was adopted from nursery preferred names (Mallet et al. 1992; Mallet 1994), cultivar checklist (Bertrand 2001) and results from molecular studies by Reed and Rinehart (2007). The first set of plants was maintained in a hoop house that was covered on all sides with 65% shade cloth (2007–2008). The second set of plants was maintained in a shadehouse open to full sun for 2 years (2007–2008) and then covered with 65% shade cloth and protected from afternoon sun by two rows of taller lilac plants (2009 and 2010). At the end of each growing season, plants in the open shadehouse (exposed to full sun or in partial shade) were moved to an enclosed shadehouse for winter protection. In early May, after the frost-free date, plants were moved back to the open shadehouse and exposed to full sun or in partial shade as before.

All plants in this study were arranged in a complete, randomized block design, with a replication of three plants per cultivar in the hoop house (shaded environment) and five plants per cultivar in the open shadehouse (full sun and partial shade environments). While the arrangement of plants in the hoop house was constant with neighbouring plants throughout the study, randomization of plants in the open shadehouse changed every year as plants were moved out of the overwintering house. All plants were fertilized in early May using a 19-5-9 Osmocote Pro™ fertilizer (Scott's-Sierra Horticultural Products Co., Maryville, OH, USA), at a rate of 143 g per pot. All plants were watered using spray stakes; plants in full sun and partial shade received additional 7-min, overhead sprinkler irrigation during mid-afternoon to enhance leaf-spot disease development. All plants were cut back in early February before bud break.

Disease development and evaluation

Development of leaf-spot symptoms on different cultivars was monitored. Disease development was attributed to natural inocula from airborne spores. Disease symptoms were characterized, and pathogens associated with different symptom patterns were isolated and identified. Disease severity was rated monthly beginning in July and ending during late September or early October. Although different symptom patterns were observed, it was impossible to separate the severity of different symptoms; however, an overall leaf-spot disease severity was evaluated. Disease-severity rating used a scale of 0–5, in which 0 = no infection, 1 = 1–10, 2 = 11–25, 3 = 26–50, 4 = 51–75 and 5 = 76–100% of leaf area showing symptoms. The highest numerical rating observed during each growing season was used as a measure of cultivar susceptibility for that season. Mean disease severity from 2 years' data was used as a measure of cultivar susceptibility under each environment (Table S1). Cultivars were categorized as resistant (R) when mean disease-severity rating was between 0.0 and 1.0, moderately resistant (MR) when disease severity was between 1.1 and 2.0, moderately susceptible (MS) when disease severity was between 2.1 and 3.0 and susceptible (S) when disease severity was between 3.1 and 5.0.

Identification of leaf-spot/leaf-blight pathogens

Isolation of pathogens from surface-disinfested leaves was performed by excising small tissue pieces from the margin of necrotic lesions and following standard isolation techniques (Dhingra and Sinclair 1995). In addition, symptomatic leaves and some stem pieces were incubated in moist chamber for 48–72 h at room temperature (21–23°C), after which they were observed under a dissecting microscope for the presence of conidiospores and/or fungal fruiting structures. A sterile needle was used to aseptically transfer representative conidiospores onto water agar and potato dextrose agar (PDA) where they were allowed to grow before isolates were subcultured into pure cultures.

Diverse fungal isolates were derived from different disease symptom types and grown on PDA. Individual

Table 1
Evaluation for cultivar susceptibility to leaf-spot diseases in garden hydrangea (*Hydrangea macrophylla*) at three environments

		Full shade		Full sun		Partial shade	
Cultivar[a]	Sub species[b]	Disease severity[c]	Disease reaction[d]	Disease severity[c]	Disease reaction[d]	Disease severity[c]	Disease reaction[d]
'Akishino Temari'	*serrata*	0.67	R	NA	NA	NA	NA
'All Summer Beauty'	*mac*	2.00	MR	2.31	MS	2.81	MS
'Altona'	*mac*	1.17	MR	2.04	MS	3.18	S
'Amagi Amacha'	*serrata*	1.67	MR	1.38	MR	NA	NA
'Amethyst'	*mac*	1.67	MR	2.50	MS	NA	NA
'Ami Pasquier'	*mac*	0.83	R	1.67	MR	1.64	MR
'Ayesha'	*mac*	0.84	R	1.50	MR	1.67	MR
'Bailmer' ('Endless Summer')	*mac*	1.34	MR	1.34	MR	3.00	MS
'Beauté Vendômoise'	*mac*	1.00	R	1.63	MR	2.82	MS
'Benigaku'	*serrata*	0.50	R	1.88	MR	2.88	MS
'Blauer Prinz' ('Blue Prince')	*mac*	1.00	R	1.17	MR	2.57	MS
'Blaumeise'	*mac*	0.33	R	2.38	MS	1.42	MR
'Blue Billow'	*serrata*	1.34	MR	1.00	R	2.35	MS
'Blue Bird'	*serrata*	0.67	R	1.42	MR	1.88	MR
'Blue Deckle'	*hybrid*	1.17	MR	1.71	MR	3.21	S
'Blue Wave'	*mac*	1.17	MR	2.92	MS	2.17	MS
'Bodensee'	*mac*	2.17	MS	1.96	MR	2.37	MS
'Bouquet Rose'	*mac*	0.50	R	1.46	MR	2.34	MS
'Charme'	*mac*	1.67	MR	1.71	MR	3.54	S
'Coerulea'	*serrata*	1.00	R	1.09	MR	2.47	MS
'Diadem'	*serrata*	1.17	MR	0.42	R	NA	NA
'Domotoi'	*mac*	2.50	MS	1.96	MS	3.50	S
'Dooley'	*mac*	1.17	MR	1.79	MR	2.32	MS
'Enziandom'	*mac*	1.50	MR	2.50	MS	2.19	MS
'Fasan'	*mac*	1.50	MR	1.17	MR	2.60	MS
'Forever Pink'	*mac*	0.50	R	2.09	MR[e]	1.42	MR
'Frillibet'	*mac*	1.00	R	2.17	MS	3.33	S
'Fuji Waterfall' ('Fujinotaki')	*mac*	1.24	MR	1.59	MR	1.75	MR
'Général Vicomtesse de Vibraye'	*mac*	1.17	MR	1.63	MR	2.75	MS
'Geoffrey Chadbund'	*mac*	1.50	MR	2.50	MS	3.44	S
'Gerda Steiniger'	*mac*	0.67	R	2.50	MS	3.36	S
'Gertrude Glahn'	*mac*	1.00	R	1.75	MR	2.53	MS
'Glowing Embers'	*mac*	0.50	R	2.00	MR	4.50	S
'Goliath'	*mac*	2.50	MS	2.67	MS	3.44	S
'Grayswood'	*serrata*	1.17	MR	2.13	MS	1.73	MR
'Hadsbury'	*mac*	0.67	R	NA	NA	NA	NA
'Hallasan'	*serrata*	1.17	MR	NA	NA	NA	NA
'Hamburg'	*mac*	0.50	R	2.96	MS	3.5	S
'Hanabi'	*mac*	1.00	R	NA	NA	NA	NA
'Harlequin'	*mac*	0.67	R	1.71	MR	3.09	S
'Hobella'	*mac*	1.33	MR	NA	NA	NA	NA
'Hokaido'	*serrata*	1.84	MR	2.04	MR	3.17	S
'Holstein'	*mac*	1.34	MR	2.63	MS	NA	NA
'Intermedia'	*serrata*	0.67	R	2.63	MS	2.25	MS
'Jōgosaki'	*mac*	0.67	R	NA	NA	NA	NA
'Kluis Superba'	*mac*	1.67	MR	1.50	MR	3.02	MS
'Komachi'	*serrata*	1.67	MR	1.25	MR	3.59	S
'Königstein'	*mac*	2.50	S	2.04	MR	3.44	S
'La France'	*mac*	0.84	R	1.59	MR	2.63	MS
'Lady in Red'	*mac*	0.67	R	NA	NA	NA	NA
'Lanarth White'	*mac*	0.50	R	0.38	R	3.17	S
'Lemon Wave'	*mac*	1.50	MR	1.29	MR	2.36	MS
'Lemon Zest'	*mac*	1.17	MR	NA	NA	NA	NA
'Libelle'	*mac*	0.67	R	2.50	MS	2.98	MS
'Lilacina'	*mac*	0.67	R	3.34	S	2.22	MS
'Madame Emile Mouillère'	*mac*	0.50	R	2.00	MR	3.29	S
'Madame Faustin Travouillon'	*mac*	1.50	MR	1.50	MR	3.00	MS
'Maréchal Foch'	*mac*	1.17	MR	1.50	MR	NA	NA
'Mariesii'	*mac*	0.67	R	NA	NA	NA	NA
'Mathilda Gütges'	*mac*	1.67	MR	2.67	MS	3.38	S

Table 1
Continued

Cultivar[a]	Sub species[b]	Full shade		Full sun		Partial shade	
		Disease severity[c]	Disease reaction[d]	Disease severity[c]	Disease reaction[d]	Disease severity[c]	Disease reaction[d]
'Merritt's Supreme'	*mac*	0.67	R	2.00	MR	2.32	MS
'Miranda'	*serrata*	1.00	R	1.21	MR	3.59	S
'Miss Belgium'	*mac*	2.00	MR	2.04	MR	3.50	S
'Miss Hepburn'	*mac*	2.33	MS	2.17	MS	2.84	MS
'Miyama-yae-Murasaki'	*serrata*	0.67	R	1.13	MR	2.00	MR
'Mousmee'	*mac*	2.00	MS	2.21	MS	3.00	MS
'Nachtigall'	*mac*	1.00	R	2.46	MS	3.00	MS
'Nigra'	*mac*	1.33	MR	1.00	R	2.69	MS
'Nikko Blue'	*mac*	1.00	R	2.34	MS	2.22	MS
'Omacha'	*serrata*	0.67	R	NA	NA	NA	NA
'Oregon Pride'	*mac*	1.00	R	1.13	MR	2.48	MS
'Otaska'	*mac*	1.00	R	1.84	MR	2.63	MS
'Parzival'	*mac*	0.50	R	1.13	MR	NA	NA
'Penny Mac'	*mac*	1.34	MR	2.59	MS	2.72	MS
'Pretty Maiden' ('Shichidanka')	*serrata*	2.17	MS	2.50	MS	3.82	S
'Preziosa'	*hybrid*	1.17	MR	2.88	MS	3.00	MS
'Seafoam'	*mac*	0.34	R	2.00	MR	1.87	MR
'Shirofuji'	*serrata*	0.50	R	NA	NA	NA	NA
'Sir Joseph Banks'	*mac*	1.00	R	NA	NA	NA	NA
'Sister Therese' ('Soeur Thérèse')	*mac*	0.83	R	3.59	S	NA	NA
'Souvenir du Président Doumer'	*mac*	1.67	MR	1.59	MR	2.42	MS
'Taube'	*mac*	1.50	MR	1.63	MR	1.62	MR
'Tödi'	*mac*	0.84	R	1.34	MR	2.61	MS
'Tokyo Delight'	*hybrid*	1.17	MR	2.92	MS	3.33	S
'Tovelit'	*mac*	1.50	MR	1.46	MR	2.38	MS
'Tricolor'	*mac*	0.83	R	1.17	MR	1.83	MR
'Trophy' ('Trophée')	*mac*	0.67	R	2.17	MS	2.37	MS
'Veitchii'	*mac*	0.50	R	1.34	MR	0.78	R

[a]Subspecies assignment for cultivars used in this article was adopted from nursery preferred names, cultivar checklist (Bertrand 2001) and results from molecular studies by Reed and Rinehart (2007).
[b]*Hydrangea macrophylla* subspecies *macrophylla* (*mac*), *serrata* and hybrid between the two subspecies.
[c]Two-year mean disease-severity readings in which 1 = 1–10, 2 = 11–25, 3 = 26–50, 4 = 51–75 and 5 = 76–100% of the plant showing disease symptoms; detailed disease-severity readings are presented in supplement Table 1. Some plants died or grew very poorly for evaluation (NA).
[d]Disease reactions categorized as resistant (R), moderately resistant (MR), moderately susceptible (MS) or susceptible (S) were based on the overall mean disease readings over 2 years in which R = 0–1.0, MR = 1.1–2.0, MS = 2.1–3.0 and S = 3.1–5.0.
[e]We designated the Forever Pink as MR under full shade although the disease severity is 2.09, which belongs to MS. Because the cultivar showed R and MR under full sun and partial shade conditions, respectively, and the disease severity is very close to MR (1.1–2.0).

isolates were evaluated for pathogenicity on healthy leaves of three cultivars 'All Summer Beauty', 'Blue Bird' and 'Nikko Blue'. Pathogenicity tests for fungal isolates were conducted using an *in vitro* assay technique that used detached leaves in moist chambers, which consisted of clear plastic containers containing paper towels soaked with sterile water (Loladze et al. 2005; Park et al. 2008). Clear plastic containers with folded paper towels drenched with sterile water were used as moist chambers for pathogenicity tests (Loladze et al. 2005; Park et al. 2008; Mmbaga et al. 2010). Four medium-sized, disease-free leaves were detached, surface disinfested and placed in each moist chamber for pathogenicity tests. These leaves were inoculated with different isolates using mycelial plugs (2-cm diameter) cut from 10- to 14-day-old cultures grown in PDA. Sterile PDA agar plugs with no mycelia were included as the control. Each half leaf was inoculated with one isolate, and the other half served as the control. Isolates proven pathogenic on any of the three hosts were re-evaluated using a spray-inoculation technique in which mycelia were macerated in sterile water and filtered through double layer of cheese cloth. The filtrates were adjusted to 1.9×10^4 propagules per ml and used to spray inoculate detached leaves, one isolate per leaf with sterile water as the negative control. Inoculated leaves were placed in clear moist chambers and incubated at 23 and 26°C. A replication of four leaves per isolate was used, and leaves were arranged in a randomized, complete block design. Re-isolation of the organisms from leaf lesions was performed to confirm the causal pathogen and fulfil Koch's Postulates (Schumann and D'Arcy 2010).

Fungal isolates obtained from infected leaves were identified using morphological features observed under a compound microscope and DNA sequence analysis (Ellis 1993; Altschul et al. 1997). Genomic DNA was extracted from conidiospores and mycelium using DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) following standard protocols. DNA amplifica-

tion was performed using polymerase chain reaction (PCR) and internal transcribed spacer (ITS) primer pairs (ITS1/ITS4) following standard PCR procedures with minor modifications (Weising et al. 1995). DNA sequence analysis was performed by the Davis Sequencing Inc. (Davis, CA, USA) (http://www.davissequencing.com). The sequences obtained were compared with all sequences of ITS region in the GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences of leaf-spot pathogens were deposited into GenBank.

Data analysis

Data analysis was performed using the SAS (Statistical Analysis Systems, Inc., Cary, NC, USA) general linear models (GLM) procedure (Schlotzauer and Littell 1987). Multiple comparisons of the mean disease severities from treatments were conducted using a series of *t*-tests between pairs of means following SAS procedures in PROC ANOVA and PROC GLM (Table S1).

Results

Disease development and evaluation

Because the plants used initially were free from leaf spot, it is inferred that subsequent leaf-spot diseases on these plants were derived from natural airborne inocula associated with the local area. In some cases, different leaf-spot symptom patterns were observed under the three environments, suggesting that different pathogens may be involved in each environment. Symptoms included necrotic lesions characterized by small, circular leaf spots with ash-coloured centres and purplish to dark-brown margins observed in all environments starting from the mid-summer (Fig. 1). These lesions began as purplish discolorations that subsequently became necrotic and sunken. Other lesions remained pinhead sized, while some enlarged and later merged to cover a large part of the leaf of some cultivars; some merged lesions maintained their characteristic circular shape (Fig. 1). Circular to irregularly shaped necrotic lesions with concentric rings of alternating brown and slightly lighter brown zones with ash-grey centres were also observed in many cultivars starting in July with increasing prevalence through September to early October (Fig. 2). Some lesions merged to cover most of the leaf surface; some lesions developed on flower petals forming well-defined brown concentric ring pattern (Fig. 2). This symptom pattern was observed in all environments, but was most prevalent in full sun to partial shade (data not shown). Another symptom pattern consisted of irregularly shaped, dark-brown lesions that sometimes merged to cover most of the leaf surface (Fig. 3). This symptom pattern was observed in all environments starting in July through October, but was most prevalent in full sun and partial shade (data not shown). Another symptom type consisting of irregularly shaped, brown, necrotic lesions with or without defined margins and chlorosis was also observed in all environments starting in July through October, but was most prevalent in full sun (Fig. 4). This symptom type occurred sporadically while symptoms shown in Figs. 1–3 were common throughout the season. Some cultivars predominantly displayed one or two symptom types, but many exhibited all lesion types; sometimes different symptoms merged and overlaid each other. Thus, it was impossible to separate different lesion types during disease monitoring/screening, and the disease-severity rating was based on overall cultivar susceptibility to all leaf-spot diseases.

Susceptibility of different cultivars to leaf-spot diseases was highly variable among cultivars, among the three environments and between years (Table 1; Table S1). Variation in temperature and rainfall between years is likely to have contributed to differences in disease severity (Fig. 5). Overall susceptibility of cultivars from 2-years' data exhibited mean disease-severity ratings, in which 51% of the cultivars were R and 41% were MR in the shaded environment (Table 1). Only a few cultivars developed significant leaf spotting in the shaded environment; of these, 'Bodensee', 'Domotoi', 'Goliath', 'Miss Hepburn', 'Mousmee' and 'Pretty Maiden' ('Shichidanka') were rated MS, while 'Königstein' was rated S (Table 1).

Many (38%) cultivars displayed high disease-severity ratings (S or MS level) under full sun, while 57 and 5% were rated as MR and R, respectively (Table 1). Disease-severity ratings were higher in partial shade, especially in 2010 when only 1% of the cultivars were rated R and 16% were rated MR (Table 1). Some cultivars were not tolerant of direct sunlight and either died or grew very poorly; these were 'Akishino Temari', 'Amagi Amacha', 'Amethyst', 'Diadem', 'Hadsbury', 'Hallasan', 'Hanabi', 'Hobella', 'Jōgosaki',

Fig. 1 Small, circular, necrotic lesions with ash-coloured centres and prominent purplish to dark-brown margins on different hydrangea (*Hydrangea macrophylla*) cultivars were associated with *Corynespora cassiicola*, *Phoma exigua* and *Cercospora* sp.

Fig. 2 Circular to irregular-shaped, necrotic lesions with concentric rings and ash-grey centre may merge or expand on different hydrangea (*Hydrangea macrophylla*) cultivars. Diseased flower petals were associated with *Corynespora cassiicola*, *Phoma exigua*, *Myrothecium roridum* and *Cercospora* sp.

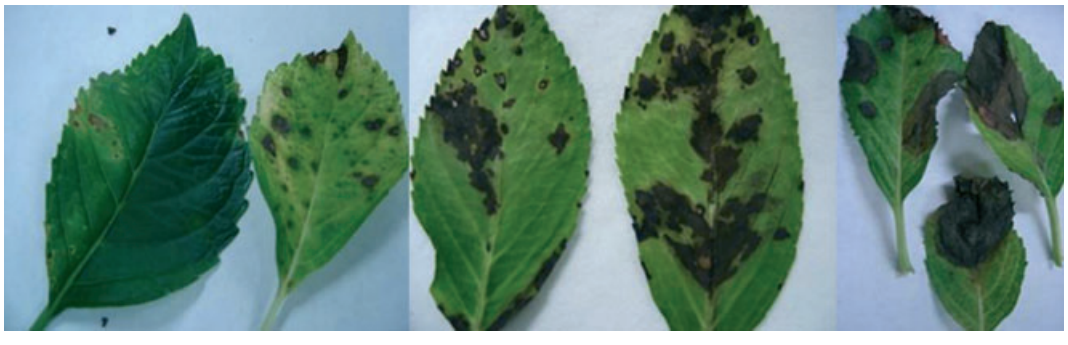

Fig. 3 Dark-brown, necrotic lesions of irregular shape with or without chlorosis on different hydrangea (*Hydrangea macrophylla*) cultivars were associated with *Coynespora cassiicola*, *Phoma exigua* and *Botrytis cinerea*

Fig. 4 Irregularly shaped, brown, necrotic lesions with or without chlorosis or margins on different hydrangea (*Hydrangea macrophylla*) cultivars were associated with *Glomerella cingulata* (*Colletotrichum gloeosporioides*), *Phoma exigua* and *Botrytis cinerea*

'Lady in Red', 'Lemon Zest', 'Maréchal Foch', 'Mariesii', 'Omacha', 'Parzival', 'Shirofuji', 'Sir Joseph Banks' and 'Sister Therese' ('Soeur Thérèse') (Table 1).

In addition to the effects of shade, full sun and partial shade on disease severity, weather influences on disease severity complicated data interpretation. Temperature and rainfall during the 2007 through 2010 growing seasons are presented in Fig. 5. Despite weather conditions that were highly favourable for leaf-spot disease (e.g., high temperatures with frequent rain showers) during 2007–2010 (Fig. 5), 10 cultivars, 'Ami Pasquier', 'Ayesha', 'Blue Bird', 'Forever Pink', 'Fuji Waterfall' ('Fujinotaki'), 'Miyama-yae-Murasaki', 'Seafoam', 'Taube', 'Tricolor' and 'Veitchii', remained R or MR under all three environments throughout this study (Table 2).

Identification of leaf-spot/leaf-blight pathogens

Fungi isolated from hydrangea leaf spots were identified as *Corynespora cassiicola*, *Cercospora* sp., *Phoma exigua*, *Myrothecium roridum*, *Glomerella cingulata* (Anamorph: *Colletotrichum gloeosporioides*), *Glomerella acutata* (Anamorph: *Colletotrichum acutata*), *Alternaria alternata* and *Botryotinia fuckeliana* (Anamorph: *Botrytis cinerea*). DNA sequence of the ITS region and morphological characterization (Ellis 1993) were used to identify leaf-spot pathogens isolated from diseased hydrangea leaves. ITS nucleotide sequence for *C. cassiicola* had 100% identity with GenBank Accession no. FJ624260 (*C. cassiicola* strain HNY35-4B) and 99% identity with other *C. cassiicola* strains in the GenBank. Furthermore, close matches were not found with any other fungi. ITS nucleotide sequence

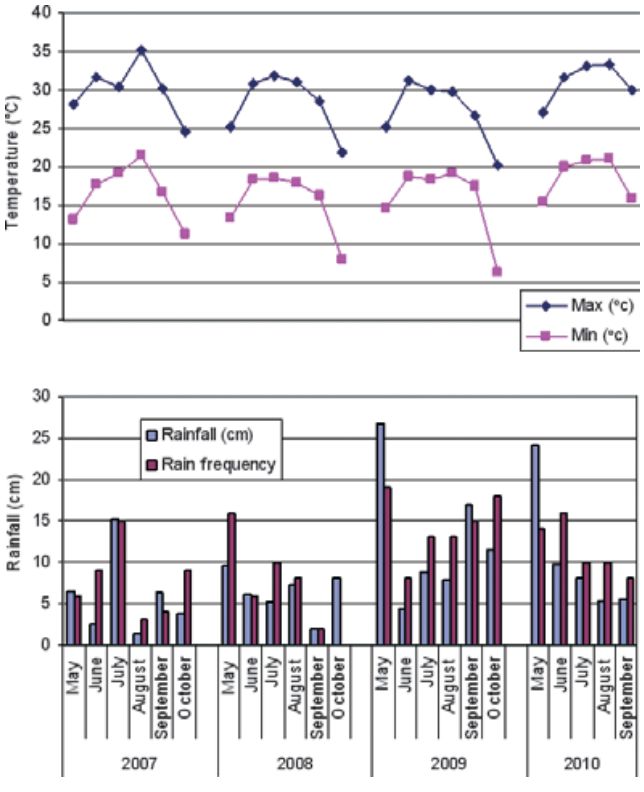

Fig. 5 Weather patterns showing temperature and rainfall in 2007 through 2010 growing seasons in McMinnville, TN, USA

for *Cercospora* sp. matched that of *Cercospora beticola* (GenBank Accession No. HQ328503), *Cercospora kikuchii* (GenBank Accession no. HM631728), *Cercospora zebrinae* (GenBank Accession no. DQ835072) and several other *Cercospora* spp. by 100%. DNA sequences of *Cercospora hydrangeae* were not found in the blast search for the *Cercospora* isolates from this study (DNA sequences for *C. hydrangeae* have not been previously deposited in the GenBank.). ITS sequences of the isolate identified as *P. exigua* showed 100% identity to GenBank Accession no. EU343173; however, sequences from this isolate also showed 100% identity to *P. multirostrata* (GenBank Accession no. GU233805) and *Phyllosticta jasmini* (GenBank Accession no. AB470839). ITS sequences from the isolate identified as *M. roridum* had closest similarity (99%) with *M. roridum* isolates (GenBank Accession nos. AJ301994 and AJ608978); other close matches (97%) were found with *M. gramineum* (GenBank Accession no. FJ235084) and *M. tongaense* (GenBank Accession no. AY254157). ITS sequences from the *G. cingulata* (*C. gloeosporioides*) isolates showed 100% identity to GenBank Accession no. DQ286192, and no close matches with any other fungi. ITS sequences of the *B. cinerea* isolates showed 100% identity with *Botryotinia fuckeliana* (anamorph: *B. cinerea*; GenBank Accession no. HM989942) and to *B. elliptica* (GeneBank Accession no. EU519207) of *Allium* spp.

Alternaria alternata and *G. acutata* were not pathogenic (data not shown), but the remaining six fungi were pathogenic and produced necrotic lesions on inoculated leaves, while sterile PDA plugs and sterile water did not produce lesions (Fig. 6). Lesions produced by different fungi were not morphologically distinct, and different fungi produced similar symptoms on inoculated leaves. While some isolates of *P. exigua* produced dark-brown lesions with concentric rings, only *M. roridium* consistently produced that symptom pattern. Spray inoculation resulted in necrotic lesions of different sizes; for example, *C. cassiicola* produced

Table 2
Cultivars of *Hydrangea macrophylla* that exhibited best performance in leaf-spot disease reactions in different environments between 2007 and 2010

		Mean disease severity[b] and disease reaction[c]							
		Mean reading (0–5 scale)[b]				Disease reaction[c]			
Cultivar	Subspecies[a]	Full shade (2007–2008)	Full sun (2007–2008)	Partial shade (2009–2010)	Overall mean (6-readings)	Full shade (2007–2008)	Full sun (2007–2008)	Partial shade (2009–2010)	Overall Mean (6-readings)
'Ami Pasquier'	*mac*	0.83	1.67	1.64	1.38	R	MR	MR	MR
'Ayesha'	*mac*	0.84	1.50	1.67	1.34	R	MR	MR	MR
'Blue Bird'	*serrata*	0.67	1.42	1.88	1.32	R	MR	MR	MR
'Forever Pink'	*mac*	0.50	2.09[d]	1.41	1.33	R	MR	MR	MR
'Fuji Waterfall' ('Fujinotaki')	*mac*	1.24	1.59	1.75	1.52	MR	MR	MR	MR
'Miyama-yae-Murasaki'	*serrata*	0.67	1.13	2.00	1.26	R	MR	MR	MR
'Seafoam'	*mac*	0.34	2.00	1.87	1.40	R	MR	MR	MR
'Taube'	*mac*	1.50	1.63	1.62	1.58	MR	MR	MR	MR
'Tricolor'	*mac*	0.83	1.17	1.83	1.27	R	MR	MR	MR
'Veitchii'	*mac*	0.50	1.34	0.78	0.87	R	MR	R	R

[a] *Hydrangea macrophylla* subspecies *macrophylla* (*mac*) and *serrata*.
[b] Disease-severity readings 1 = 1–10, 2 = 11–25, 3 = 26–50, 4 = 51–75 and 5 = 76–100% of the plant showing disease symptoms.
[c] Disease reactions categorized as resistant (R), moderately resistant (MR), moderately susceptible (MS) or susceptible (S) were based on the mean disease readings derived from multiple years in which R = 0–1.0, MR = 1.1–2.0, MS = 2.1–3.0 and S = 3.1–5.0.
[d] We designated the 'Forever Pink' as MR under full shade although the disease severity is 2.09, which belongs to MS. Because the cultivar showed R and MR under full sun and partial shade conditions, respectively, and the disease severity is very close to MR (1.1–2.0).

Fig. 6 Necrotic lesions reproduced by different fungi on inoculated hydrangea (*Hydrangea macrophylla*) leaves

large lesions on 'Nikko blue' and 'All Summer Beauty' and smaller circular lesions on 'Blue Bird', while *Cercospora* sp. produced small, circular lesions on 'Nikko Blue' and 'All Summer Beauty' and no lesions on 'Blue Bird.' The representative ITS DNA sequences of the different fungi isolated from hydrangea leaf spots have been deposited in the GenBank with the following GenBank Accession numbers: HQ845386 for *C. cassiicola*, HQ845387 for *Cercospora* sp., HQ845385 for *G. cingulata* (anamorph *C. gloeiosporoides*), HQ845384 for *P. exigua*, HQ845388 for *B. cinerea*, HM215150 for *M. roridium*, HQ878371 for *A. alternata* and HQ878372 for *G. acutata*.

More than one fungus was isolated from each symptom type, *C. cassiicola*, *Cercospora* sp., *P. exigua* and *G. acutata* were isolated from small, circular, necrotic leaf spots with ash-coloured centres and purplish to dark-brown margins (Fig. 1). *Corynespora cassiicola*, *M. roridum*, *C. gloeiosporoides* and *P. exigua* were isolated from the large circular to irregular-shaped, necrotic lesions with concentric rings (Fig. 2). *Corynespora cassiicola*, *P. exigua*, *B. cinerea* and *A. alternata* were isolated from dark-brown, necrotic lesions of irregular shape with or without chlorosis as shown in Fig. 3. *Glomerella cingulata* (anamorph *C. gloeosporioides*) and *B. cinerea* were isolated from angular to circular, brown, necrotic lesions presented in Fig. 4. Pathogenicity of each of these fungi was confirmed by re-isolation of the same fungus from the lesions of inoculated leaves. Of >100 isolates obtained over the course of this study, 55% were of *C. cassiicola*, 20% *Cercospora* sp., 5% *Phoma*, 5% *M. roridium*, 5% *B. cinerea*, 5% *G. cingulata* (*C. gloeosporioides*), 3% *A. alternata* and 2% *G. acutata*.

When stem pieces from plants with dieback were incubated in a moist chamber for 48 h, abundant spores of *C. cassiicola* and *A. alternata* were observed, but the *A. alternata* was not pathogenic. When infected leaves were incubated in the moist chamber for 48 h, abundant spores of both *C. cassiicola* and *Cercospora* spp. developed from the small circular leaf spots shown in Fig. 1, but spores of these two species were distinguished morphologically even when the two fungi co-occurred (Ellis 1993; Barnett and Hunter 1998). Abundant conidiospores of *C. cassiicola* developed from large lesions shown in Figs 2 and 3, while blister-like protrusions of fruiting bodies (arcevuli) containing abundant spores of *G. cingulata* (anamorph *C. gloeosporioides*) (Barnett and Hunter 1998) developed from large, lighter brown, necrotic lesions (Figs 2 and 4). Of the six pathogens, *C. cassiicola* was the most aggressive in pathogenicity tests, causing leaf necrosis in 3 days as compared to 4–5 days for the other pathogens. Some isolates of *C. cassiicola* caused large lesions that covered most of the leaf while other isolates caused smaller lesions (Fig. 6).

Discussion

Garden hydrangea are best adapted to shaded environments (Dirr 2004), but that environment also favours powdery mildew outbreaks (Mmbaga et al. 2008). Studies by Li et al. (2008) showed that Cercospora leaf-spot disease severity was lower in higher shade environment and suggested that selection/screening for resistant cultivars should be performed under full sun. Results from this study show that both full sun and partial shade were good environments for resistance screening of hydrangea leaf-spot diseases. However, some cultivars did not tolerate direct sunlight and were not evaluated; such cultivars would not be suitable for the landscape use. Most cultivars evaluated in this study were rated R or MR in shaded environment, and only a few cultivars were rated S or MS, as previously reported by Li et al. (2008). This result indicates that disease-resistant hydrangea cultivars and a shaded environment can be used as effective components of integrated disease management for hydrangea leaf-spot diseases. Ten cultivars were clearly resistant in all environments; these cultivars are valuable resources for nursery growers, landscape industry and hydrangea breeding programs (Table 2). Of the cultivars rated R or MR under all environments, only 'Miyama-yae-Murasaki' and 'Veitchii' are also resistant to powdery mildew (Mmbaga et al. 2008; Windham et al. 2011). Cultivars rated R or MR to both leaf-spot diseases and powdery mildew in shaded environment are also valuable resources for nursery and landscape industry; these were 'Akishino Temari', 'Diaden', 'Miyama-yae-Muraski', 'Omacha', Shirofuji' and 'Veitchii' (Windham et al. 2011; Tables 1 and 2).

Leaf-spot pathogens survive on previously infested plant debris and dormant buds (Hagan and Mullen 2001). Thus, inoculum build-up over time may have contributed to the increasing disease levels in each environment. Such situations often result in yearly leaf-spot disease outbreaks (Hagan and Mullen 2001).

Based on inoculum availability alone, leaf-spot disease severity was expected to increase progressively between 2007 and 2010. However, environmental differences in shade, partial shade and full sun and differences in weather conditions in different years also impacted disease severity. While 2007 summer was dry and unusually hot, the cooler temperatures during 2008 and 2009 were less favourable to hydrangea leaf-spot diseases, and the increased rain showers during 2009 were favourable to pathogen spread. High temperatures during July through September 2010 were similar to 2007, but the combination of high temperatures and frequent rainfalls in 2010 favoured pathogen spread and high disease severity. Rainfall was highest in 2009 when temperatures were generally moderate (Fig. 5), and the disease-severity rating ranged from approximately 0.6–4.5 as compared to 1–5 in 2010 when both temperature and rainfall were more favourable for disease development. Results from this study provide additional evidence that the complex interactions among environmental factors can influence disease severity.

Results from our studies support previous reports that various pathogens cause hydrangea leaf spots (Hagan and Mullen 2001; Williams-Woodward and Daughtrey 2001). Of the fungi that occurred naturally at the study site (McMinnville, TN, USA), pathogenicity of *C. cassiicola*, *Cercospora* sp., *M. roridum*, *G. cingulata* (Anamorph: *C. gloeosporioides*), *P. exigua* and *B. cinerea* was confirmed on three cultivars. These six fungal pathogens have been reported to cause leaf-spot disease wherever hydrangea are grown. Symptoms resembling those presented in Fig. 1 have been described for Cercospora leaf spot, also known as 'frogeye leaf spot' caused by *C. hydrangeae* (Hagan and Mullen 2001; Vann 2010; http://www.htpp//plantpath.caes.uga.edu/extension) as well as Corynespora leaf spots (Wolcan et al. 2005; Schlub and Smith 2007). In our study, three fungal pathogens were isolated from leaves displaying this symptom: *C. cassiicola*, *Cercospora* sp. and *P. exigua*. After infected leaves were incubated in moist chamber for 48 h, spores of both *C. cassiicola* and *Cercospora* sp., but not *P. exigua*, were observed on the lesions. Observations from this study raise questions as to whether disease outbreaks referred to as Cercospora leaf spot are caused by *Cercospora* sp., *C. cassiicola* and *P. exigua* or a mixture of these pathogens. In our observations, once *Corynespora* is introduced into a plant, yearly outbreaks of the disease are likely to occur. Thus, continued studies are needed to assess the reaction of individual hydrangea cultivars to individual fungal pathogens in a controlled environment. Identification of resistant cultivars is essential for hydrangea breeding programs for cultivars that are resistant to major leaf-spot pathogens, such as *C. cassiicola* and *Cercospora* spp.

In this study, four fungal pathogens (*C. cassiicola*, *P. exigua*, *C. gloeosporioides* and *M. roridum*) were isolated from symptoms that resembled those described for anthracnose or 'target-leaf spot' caused by *C. gloeosporioides* (Hagan and Mullen 2001; Fig. 2). However, *C. gloeosporioides* was recovered from only 5% of leaves displaying this symptoms pattern, which agrees with the sporadic occurrences reported in the literature (Hagan and Mullen 2001). Interestingly, only *M. roridum* consistently reproduced lesions with concentric rings in pathogenicity tests. While *M. roridum* was first reported in the USA in 2010 (Mmbaga et al. 2010), this pathogen has been associated with wide host range causing leaf spots/blight and reducing seed germination (Dake 1980; Ravishankar and Mamatha 2005; Mangandi et al. 2007).

Phoma exigua has been reported as a pathogen of hydrangea in the USA, and it caused severe defoliation and had a detrimental impact on the aesthetic value of the diseased plants in Italy (Garibaldi et al. 2006). In our study, the occurrence of this pathogen was low, and its impact on plant aesthetics could not be evaluated because its presence was a part of a disease complex. A previous report indicates that *B. cinerea* causes grey mould on hydrangea during warm and wet weather (Hagan and Mullen 2001). Although *B. cinerea* has wide distribution, it was only found at low frequency on hydrangea during July. While crowded plants and high humidity can create favourable environment for leaf-spot pathogen activity, high temperatures are favourable to hydrangea leaf-spot disease development (Hagan and Mullen 2001; Hagan et al. 2004).

Glomerella acutata is recognized as a cosmopolitan pathogen recovered from a wide range of plants, and it is often found in close association with other *Colletotrichum* species (Guerber and Correll 2001). However, *G. acutata* was not pathogenic on the three hydrangea cultivars. Similarly, *A. alternata* has been reported to cause dark leaf spots on some hydrangea cultivars in Italy (Garibaldi et al. 2007), but in our studies, disease symptoms were not reproduced, possibly because the cultivars used for pathogenicity tests may be resistant to this pathogen. *Alternaria alternata* are also common saprophytes on most plants and plant debris (Rotem 1994). Thus, it is possible that the *A. alternata* isolates from our studies were saprophytes. More studies are needed to determine the role of different pathogens in the leaf-spot disease complex of hydrangea.

Acknowledgements

We would like to thank Ms Teri Simons and Rotsitsa Owens for their efforts in pathogen isolations and pathogenicity tests; Mr Terry Kirby for plant maintenance; Dr Ned B. Klopfenstein for helpful comments on an earlier version of this manuscript; Dr Sandra Reed for providing the plants materials used in this study and editorial remarks on the results presentation. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Tennessee State University or the U.S. Department of Agriculture.

References

Altschul SF, Madden TL, Schafferr AA, Zhang J, Zhang Z, Miller W, Lipman DJ. (1997) Gapped BLAST and PSI-BLAST: a new

generation of protein database search programs. *Nucleic Acids Res* **25**:3389–3402.
Bailey DA. (1989) *Hydrangea Production*. Portland, OR, USA, Timber Press.
Barnett HL, Hunter BB. (1998) *Illustrated Genera of Imperfect Fungi*. St Paul, MN, USA, APS Press.
Bertrand H. (2000) Management and knowledge of the *Hydrangea* collection of Angers. Morphological characters and data analysis. *Acta Hortic* **508**:173–178.
Bertrand H. (2001) *Preliminary Hydrangea Checklist*. Angers, France, Institut National d'Horticulture.
Dake GN. (1980) Effect of *Myrothecium roridum* on the germination of cotton seed. *Indian Phytopathol* **33**:591–593.
Dhingra OD, Sinclair JB. (1995) *Basic Plant Pathology Methods*, 2nd edn. Boca Raton, FL, USA, CRC Press.
Dirr MA. (2004) *Hydrangeas for American Gardens*. Portland, OR, USA, Timber Press.
Ellis MB. (1993) *Dematiaceous Hyphomycetes*. Oxon, UK, CAB International.
Garibaldi A, Gilardi G, Minerdi D, Gullino ML. (2006) First report of leaf-spot caused by *Phoma exigua* on *Hydrangea macrophylla* in Italy. *Plant Dis* **90**:1113.
Garibaldi A, Gilardi G, Frati S, Gullino ML. (2007) First report of leaf-spot caused by *Alternaria alternata* on *Hydrangea macrophylla* in Italy. *Plant Dis* **91**:767.
van Gelderen CJ, van Gelderen DM. (2004). *Encyclopedia of Hydrangeas*. Portland, OR, USA, Timber Press.
Guerber JC, Correll JC. (2001) Characterization of *Glomerella acutata*, the teleomorph of *Colletotrichum acutatum*. *Mycologia* **93**:216–229.
Hagan AK, Mullen JM. (2001) Diseases of Hydrangea. Alabama Cooperative Extension System, ANR 1212.
Hagan AK, Olive JW, Stephenson J, Rivas-Davilla ME. (2004) Impact of application rate and interval on the control of powdery mildew and cercospora leaf spot on bigleaf hydrangea with azoxystrobin. *J Environ Hort* **22**:58–62.
Haworth-Booth M. (1984) *The Hydrangeas*, 5th edn. London, UK, Constable and Company.
Li Y, Windham MT, Trigiano RN, Reed SM, Spiers JM, Rinehardt TA. (2008) Effects of shading on Cercospora leaf spot in bigleaf hydrangea. *Proc Southern Nurs Assoc Res Conf* **53**:35–36.
Loladze A, Garland Campbell KA, Chen X. (2005) Development of a detached leaf assay for stripe rust resistance screening. Agronomy Abstracts. http://crops.confex.com/crops/2005am/techprogram/P3298.HTM
Mallet C. (1994) *Hydrangeas: Species and Cultivars*, Vol. 2. Varengeville, France, Centre d'Art Floral.
Mallet C, Mallet R, van Trier H. (1992) *Hydrangeas: Species and Cultivars*, Vol. 1. Varengeville, France, Centre d'Art Floral.
Mangandi JA, Seijo TE, Peres NA. (2007) First report of *Myrothecium roridum* causing Myrothecium leaf spot on *Salvia* spp. in the United States. *Plant Dis* **91**:772.
McClintock E. (1957) A monograph of the genus *Hydrangea*. *Proc Calif Acad Sci* **29**:147–256.
Mmbaga MT, Reed SM, Windham MT, Yonghao L, Rinehart TA. (2008) Disease resistance in commercial cultivars of *Hydrangea macrophylla*. *Phytopathology* **98**:S108.
Mmbaga MT, Li Y, Kim M-S. (2010) First report of *Myrothecium roridum* in the United States. *Plant Dis* **94**:1266.
Mmbaga MT, Windham MT, Li YR, Sauvé RJ. (2009) Fungi associated with naturally occurring leaf spots and leaf bights in *Hydrangea macrophylla* in McMinnville Tennessee. *Proc Southern Nurs Assoc Res Conf* **54**:1–5.
Park D-S, Sayler RJ, Hong YG, Nam MH, Yang Y. (2008) A method for inoculation and evaluation of rice sheath blight disease. *Plant Dis* **92**:1.
Ravishankar RV, Mamatha T. (2005) Seedling diseases of some important forest tree species and their management. In: Working Papers of the Finnish Forest Research Institute 11. pp. 51–63. http://www.metla.fi/julkaisut/workingpapers/2005/mwp011.htm
Reed SM, Rinehart TA. (2007) Simple sequence repeat marker analysis of genetic relationships within *Hydrangea macrophylla*. *J Am Soc Hort Sci* **132**:341–351.
Rotem J. (1994) *The Genus Alternaria: Biology, Epidemiology and Pathogenicity*. St Paul, MN, USA, APS Press.
SAS Institute. (1990) *SAS*/STAT Users Guide, Version 9. Cary, USA, Statistical Analysis Systems Institute, Inc.
Schlotzauer SD, Littell RC. (1987) *SAS System for Elementary Statistical Analysis*. Cary, USA, Statistical Analysis Systems Institute, Inc.
Schlub RL, Smith LJ. (2007) Diagnostic features of *Corynespora cassiicola* and its associated diseases. NPDN National Meeting Abstract 39. http://www.plantmanagementnetwork.org/proceedings/npdn/2007
Schumann GL, D'Arcy CJ. (2010) *Essentials Pant Pathology*, 2nd edn. St Paul, USA, SAPS Press.
Sinclair WA, Lyon HH, Johnson WJ. (1987) *Diseases of Trees and Shrubs*. Ithaca, USA, Cornell University Press.
Vann S. (2010) *Cercospora Leaf Spot of Hydrangea*. University of Arkansas, FSA7570-PD-11-09N. Internet Resource: www.arhomeandgarden.org/News/Arkansas_garden/ (verified on May 31, 2011).
Vintage Gardens. (2008) Hydrangea care, culture and historical background. http://www.vintagegardens.com/hydro_care.htm
Weising K, Nyborm H, Wolff K, Meyer W. (1995) *DNA Fingerprinting in Plants and Fungi*. Boca Raton, FL, USA, CRC Press, 472 pp.
Williams-Woodward JL, Daughtrey ML. (2004) Hydrangea diseases. In: Jones RK, Benson MD. (eds) *Diseases Compendium of Nursery Crop Diseases*. St Paul, MN, American Phytopathological Society Press, pp 191–194.
Windham MT, Reed SM, Mmbaga MT, Windham AS, Li Y, Rinehart TA. (2011) Evaluation of powdery mildew resistance in *Hydrangea macrophylla*. *J Environ Hort* **29**:60–64.
Wolcan SM, Dal Bello GM, Sisterna M. (2005) Cercospora leaf spot on *Impatiens* spp. in Argentina. *New Dis Rep* **12**:17.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Leaf-spot disease evaluation in *Hydrangea macrophylla* grown in different environments in McMinnville, TN, USA 2007–2010.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.