Genetics and resistance / Génétique et résistance

Assessment of resistance components of bigleaf hydrangeas (*Hydrangea macrophylla*) to *Erysiphe polygoni* in vitro

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Abstract: Powdery mildew caused by *Erysiphe polygoni* is one of the major foliar diseases on bigleaf hydrangeas (*Hydrangea macrophylla*). To determine resistance components of bigleaf hydrangea to powdery mildew, fungal development and host necrotic cells were compared on six bigleaf hydrangea cultivars using a detached leaf disk bioassay. Germination percentages of conidia were not significantly different among cultivars. However, percent geminated conidia with secondary germ tubes (GCSGT), percent necrotic host cells, infection efficiency, latent period, and sporulation were significantly different among cultivars. In general, 'Veitchii' was resistant, 'Nikko Blue' was susceptible, and 'Madame Emile Mouillere', 'Forever Pink', 'Lilacina', and 'Holstein' were intermediate. Necrotic cells were macroscopically visible in all cultivars regardless of assigned resistance levels. Significantly more necrotic cells were detected in resistant cultivar 'Veitchii' compared with other cultivars. These results suggest that hypersensitive reaction is not a qualitative trait of resistance, but the frequency of necrotic cells could be one of several resistance components that contribute to restrain fungal growth and colony development. Additionally, percent GCSGT, infection efficiency, latent period, and sporulation could be used to evaluate partial resistance in bigleaf hydrangea to powdery mildew using a detached leaf disk assay.

Key words: powdery mildew, *Hydrangea macrophylla*, *Erysiphe polygoni*, partial resistance.

Résumé : L'oïdium causé par *Erysiphe polygoni* est une des principales maladies foliaires qui s'attaquent à l'hortensia commun (*Hydrangea macrophylla*). Afin de déterminer les composantes de résistance de l'hortensia commun à l'oïdium, le développement fongique et les cellules nécrotiques hôtes ont été comparés sur six cultivars d'hortensia commun à au moyen d'une épreuve biologique sur disques foliaires détachés. Chez les cultivars, les taux de germination des conidies ne variaient pas significativement. Toutefois, le taux de conidies germées avec tubes germinatifs secondaires (GCSGT), le taux de cellules nécrotiques hôtes, la période de latence et la sporulation variaient significativement d'un cultivar à l'autre. En règle générale, 'Veitchii' était résistant, 'Nikko Blue' était réceptif et 'Madame Emile Mouillere', 'Forever Pink', 'Lilacina' ainsi que 'Holstein' se situaient à peu près à mi-chemin entre les deux précédents quant à la résistance. Les cellules nécrotiques étaient visibles à l'oeil nu chez tous les cultivars, indépendamment des niveaux de résistance. Un nombre plus important de cellules nécrotiques ont été détectées chez le cultivar résistant 'Veitchii', comparativement aux autres cultivars. Ces résultats suggèrent qu'une réaction d'hypersensibilité n'est pas une caractéristique qualitative de la résistance, mais la fréquence des cellules nécrotiques pourrait être une des composantes de résistance qui contribue à restreindre la croissance fongique et le développement des colonies. De plus, le taux de GCSGT, l'efficacité d'infection, la période de latence et la sporulation pourraient être utilisés pour évaluer la résistance horizontale à l'oïdium chez l'hortensia commun au moyen d'une épreuve biologique sur disques foliaires détachés.

Mots-clés : oïdium, *Hydrangea macrophylla*, *Erysiphe polygoni*, résistance horizontale.

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Introduction

Bigleaf hydrangea (*Hydrangea macrophylla* (Thumb.) Ser.) is one of the most popular species of *Hydrangea* and is grown as both a landscape and greenhouse plant. Powdery mildew, caused by *Erysiphe polygoni* DC., is a foliar disease of bigleaf hydrangea affecting inflorescences and causing yellow blotches on leaves (Daughtrey et al. 1995; Dirr 2004). Plants grown in greenhouses or in shade are particularly susceptible to the disease (Dirr 2004; Williams-Woodward and Daughtrey 2001).

Powdery mildew fungi are obligate biotrophic plant parasites that invade the epidermal cells of hosts. After conidia land on the host surface, the formation of germ tubes and appressoria is essential for penetration of the host cuticle and cell wall. Formation of haustoria to absorb nutrients and support hyphal growth is necessary for biotrophy establishment. Fungal development can be affected by host resistance at many stages of the infection process (Niks and Rubiales 2002), such as haustorium formation (Martinez et al. 2004) and hyphal growth (Carver and Carr 1978; Li et al. 2006). Hypersensitive response (HR) that is characterized by rapid death of invaded cells is one of the major resistance mechanisms to restrict fungal development (Adam and Somerville 1996; Cohen and Eyal 1988; Kuzuya et al. 2006; Prats et al. 2007; Sameshima et al. 2004; Vorwerk et al. 2007). Restriction or inhibition of hyphal growth on resistant cultivars has been reported in several powdery mildew pathosystems (Floris and Alvarez 1996; Huang et al. 1998; Kuzuya et al. 2003; Poulter et al. 2003). On the other hand, non-hypersensitive resistance responses can also restrict disease development and reduce spore production by preventing haustorium formation (Niks 1986) or by restricting colony growth (Carver and Carr 1978; Li et al. 2006). Partial resistance is characterized as continuous variation in fungal sporulation (Niks and Rubiales 2002; Parlevliet 1992). Infection efficiency, latent period, and sporulation have been used as components to evaluate partial resistance to plant diseases (Diaz-Lago et al. 2003; Li et al. 2006; Lindhout et al. 1994; Parlevliet 1989; Viljanen-Rollinson et al. 1998).

Variation in resistance to *E. polygoni* has been reported previously among bigleaf hydrangea cultivars (Dirr 2004; Li et al. 2009). Based on the evaluation using the percent leaf surface affected in a field trail, cultivars 'Nikko Blue' and 'Holstein' were susceptible, cultivar 'Veitchii' was resistant with no visible symptoms, and cultivars 'Forever Pink', 'Madame Emile Mouillere', and 'Lilacina' were intermediate (Dirr 2004). Comparisons of the fungal development on detached leaves showed that host cell necrosis occurred earlier and fungal development was restricted in the resistant cultivar 'Veitchii' compared with the susceptible cultivar 'Nikko Blue'. However, haustoria were formed under primary appressoria and initiated secondary germ tubes, and necrosis of infected host cells was observed in both cultivars (Li et al. 2009). The objectives of this study were to (i) quantitatively compare the fungal development including spore germination, secondary germ tube initiation, necrotic host cell, infection efficiency, latent period, and sporulation on detached leaves of six cultivars with different levels of resistance and (ii) to characterize resistance components to powdery mildew in bigleaf hydrangeas.

Materials and methods

Plant materials and inoculation

Six bigleaf hydrangea cultivars, 'Forever Pink', 'Holstein', 'Lilacina', 'Madame Emile Mouillere', 'Nikko Blue', and 'Veitchii', were used in the experiments. These cultivars were selected because of their differences in resistance to powdery mildew (Dirr 2004). Two-year-old bigleaf hydrangea plants purchased from Hydrangeas Plus of VanHoose Enterprises, LLC, Aurora, Ore., were potted in 3 gallon containers and grown in a greenhouse under a 50% shade cloth at the Plant Sciences Unit of the East Tennessee Research and Education Center in Knoxville, Tenn. Plants were watered once every two days and fertilized with Peters Professional Soluble Plant Food (The Scotts Company, Maryville, Ohio) once a month.

Fully expanded healthy leaves were collected from one plant for each cultivar and washed in running tap water for 1 min. Leaf disks were cut using a 1.2 cm diameter cork borer and placed with the abaxial surface up on two layers of moistened P8-creped filter paper (Fisher Scientific, Pittsburgh, Pa.) in 9 cm Petri dishes. There was one leaf disk for each cultivar in each Petri dish and three Petri dishes for each inoculation. Powdery mildew inoculum was collected from naturally infected hydrangea plants at the Otis L. Floyd Nursery Research Center at McMinnville, Tennessee, and maintained on bigleaf hydrangea 'Nikko Blue' plants in a greenhouse at the campus of the University of Tenn. at Knoxville. Inoculation was conducted by blowing conidia of *E. polygoni* from infected leaves in the settling tower as previously described (Li et al. 2009). The spore density on inoculated leaf disks was maintained at 200–300 conidia/cm^2 by adjusting the number of air blasts during the inoculation. After inoculation, the Petri dishes were sealed with parafilm and incubated at 21±2 °C with a continuous photoperiod. Light was provided by four 40 watt residential fluorescent bulbs located 45 cm above the leaf disks.

Spore germination and secondary germ tube formation

To determine the effects of host resistance on percent spore germination, inoculated leaf disks were dipped in 5% cellulose in acetone for 1 min at one day after inoculation (DAI) to prevent loss of ungerminated conidia during the staining process and put on a piece of filter paper. After the acetone evaporated, cellulose membranes were peeled off the leaf disks, affixed onto glass slides, stained with 0.01% aqueous trypan blue, covered with glass coverslips, and sealed with permount (Fisher Scientific, Fair Lawn, N.J.). Spore germination was examined by observing 100 conidia on each membrane under a compound microscope (Nikon Instruments, Melville, N.Y.). Conidia with primary appressoria or a primary germ tube longer than the width of conidia were considered as germinated conidia (Fig. 1).

To compare the percent germinated conidia with secondary germ tubes (GCSGT) at 2 DAI, inoculated leaf disks were cleared and stained by using the method described by Schiffer et al. (1997). Leaf disks were placed on two-layers of P8-creped filter paper saturated with a solution of 0.15%

Fig. 1. Micrographs of (A) ungerminated and (B) germinated conidia with a primary germ tube (PGT) and primary appressoria (PAP) of *Erysiphe polygoni* on cellular membranes that were peeled off from inoculated bigleaf hydrangea leaves 24 h after inoculation. Scale bars represent 10 μm.
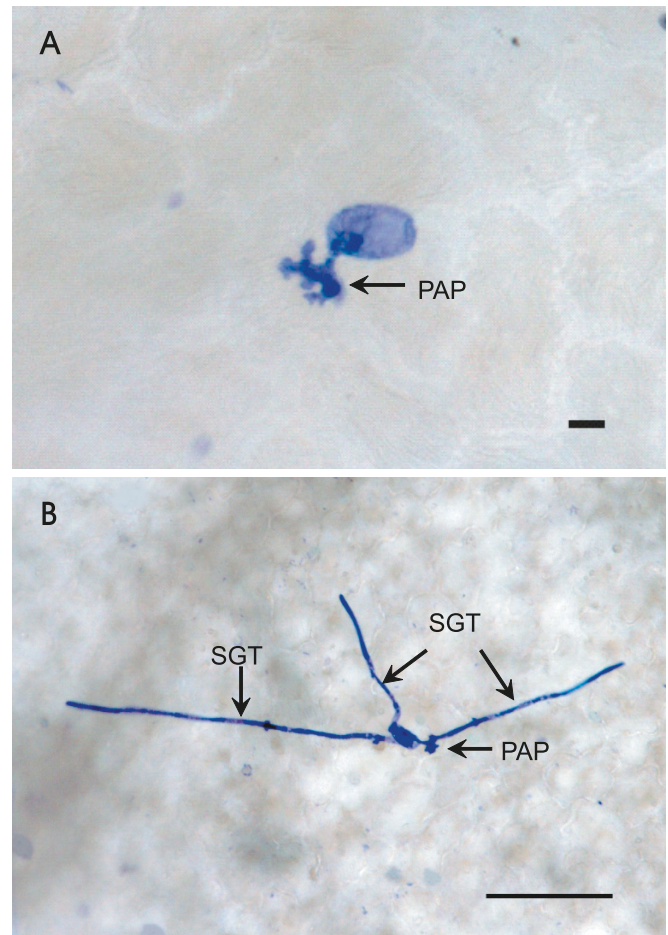

Fig. 2. Micrographs of germinated conidia primary appressoria (PAP) (A) without or (B) with secondary germ tubes (SGT) of *Erysiphe polygoni* on bigleaf hydrangea leaves 2 days after inoculation. Scale barsrepresent 10 μm and 100 μm in (A) and (B), respectively.
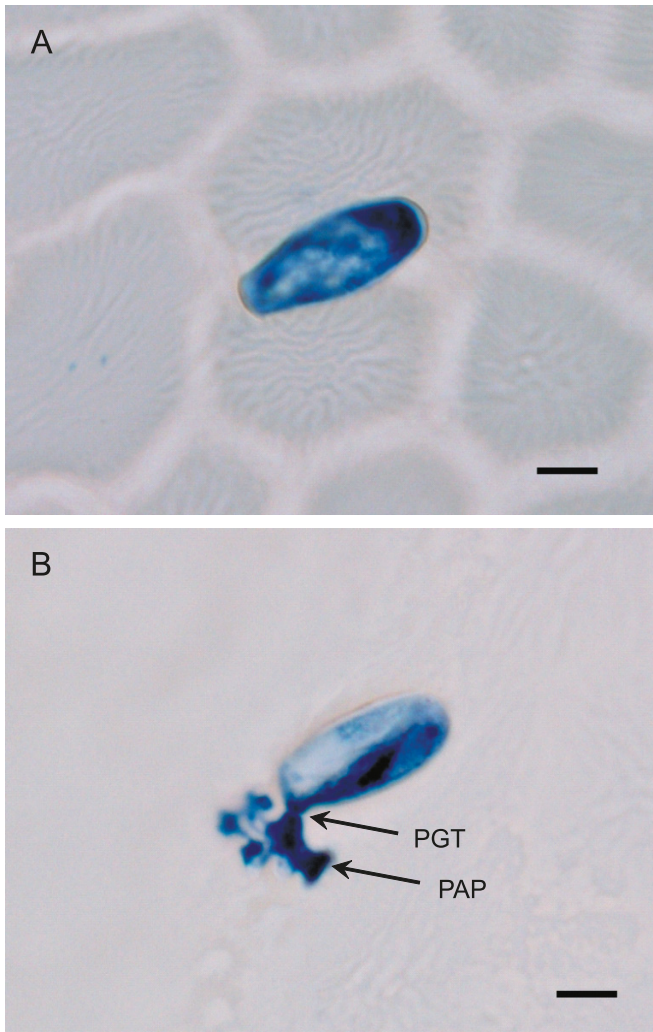
trichloroacetic acid in a chloroform–alcohol mixture of 1:4 (*v/v*) in 6 cm diameter Petri dishes at room temperature. Filter papers in the Petri dishes were changed once at 24 h during a 48 h period. Cleared leaf disks were stained with 0.6% coomassie brilliant blue R-250 in 10% trichloroacetic acid for 30 s, washed with distilled water, mounted in 50% glycerol on glass slides, and examined under the compound microscope (200×). One hundred germinated conidia were randomly examined on each leaf disk and GCSGT were recorded for each leaf disk (Fig. 2).

Dead infected cells

Cellular responses of host epidermal cells to the fungal penetration were examined after staining leaf disks with trypan blue as described by Sillero and Rubiales (2002). Inoculated leaf disks were sampled 5 DAI and placed on two layers of filter paper moistened with a acetic acid–ethanol fixation solution for 30 min. Fixed disks were stained with 0.05% trypan blue in a lactopahenol–ethanol mixture (1:2

(*v:v*)) in a boiling water bath for 3 min. Cooled stained leaf disks were cleared on filter paper soaked with saturated chloral hydrate for 48 h at room temperature with replacement of filter papers and saturated chloral hydrate at 24 h. The disks were mounted in 50% glycerol on glass slides and coverslips were sealed with permount. Percent of dead infected cells (PDIC) were assessed by observing 100 to 130 epidermal cells infected with haustoria in each leaf disk using a compound bright-field microscope (200×). A necrotic cell was defined as a whole epidermal cell or a cell with more than 50% of its cell wall stained heavily by trypan blue.

Infection efficiency

Infection efficiency (IE), in the present study, was defined as the percent of inoculated conidia that formed colonies 7 DAI (Parlevliet 1979). Seven DAI was chosen because mildew colonies were fully developed and easily differentiated at this time. A mildew colony was defined as a germinated conidium that formed more than five branched hyphae. The number of powdery mildew colonies was counted for each disk using a stereo microscope (30×). The

Table 1. Comparisons of percent germinated conidia with secondary germ tubes (PGCSGT), percent infected necrotic cells (PDIC), infection efficiency (IE), latent period (LP), and sporulation (SP) of powdery mildew caused by *Erysiphe polygoni* in six bigleaf hydrangea cultivars.

Cultivar	PGCSGT (%)	PDIC (%)	IE (%)	LP (day)	SP (conidia/disk)
'Nikko Blue'	76.0 a	21.2 b	26.5 a	6 b	8625 a
'Forever Pink'	61.2 b	21.0 b	23.3 a	6 b	2417 b
'Lilacina'	56.1 b	22.8 b	13.8 b	6 b	3458 b
'Madame Emile Mouillere'	49.1 b	28.8 b	14.9 b	6 b	3375 b
'Holstein'	30.0 c	21.5 b	6.0 c	6 b	1729 b
'Veitchii'	33.2 c	65.6 a	1.6 d	14 a	61 c

Note: Analysis of variance was conducted using arcsine-transformed data for PGCSGT, PDIC, and IE and using square-root-transformed data for SP. Means followed by the same letter in a column for each variable are not significantly different from each other at the $P = 0.05$ level of the Tukey's minimum significant difference.

inoculum density (conidia/cm^2) for each inoculation was determined by placing a gridded glass slide in the settling tower and conidia in the grids were counted using a stereo microscope (50×). Infection efficiency on each leaf disk was calculated using the equation

$$IE\ (\%) = C/(S \times A),$$

where *IE* is infection efficiency, *C* is the number of colonies on a leaf disk, *S* is the number of conidia inoculated on 1 cm^2 area, and *A* is the area of a leaf disk.

Latent period

Inoculated leaf disks were examined daily for conidiophores and conidia formation using a stereo microscope (60×). Latent period was defined as the number of days between inoculation and the first appearance of sporulation, which was a modification of the definition described by Parlevliet (1979). Three leaf disks per cultivar were examined for the formation of conidiophores and conidia, and the experiment was repeated four times.

Sporulation

Sporulation was defined as the number of conidia produced per leaf disk 14 DAI. Each inoculated leaf disk was transferred into 25 mL screw-top tubes containing 8 mL of 0.1% Tween 20 solution 14 DAI and agitated using a vortex mixer for 1 min. After removing leaf disks, tubes were centrifuged for 10 min at 1000*g*. After discarding the supernatant, pellets in the tubes were resuspended in 0.5 mL of distilled water. The number of conidia in the suspensions was counted using a hemocytometer and a compound microscope (200×).

Statistical analysis

Experiments were arranged in randomized complete block designs (RCBD) with subsamplings, except the necrotic cell experiment that was conducted using RCBD without subsampling. The inoculation in a settling tower was considered as a blocking random factor and six cultivars were randomly assigned in a block as treatments. Three leaf disks that were placed separately in three Petri dishes for each cultivar per inoculation were considered as three subsamples. All experiments were repeated four times except the necrotic cell experiment that was repeated three

times. Each experiment had its own separate set of leaf disks. Before statistical analyses, the arcsine-transformation (Hoshmand 2006) was conducted for the percent data of spore germination, PGCSGT, PDIC, and IE, and square root transformation was conducted for sporulation data (May and Trigiano 1991). Analysis of variance (ANOVA) was conducted using the general linear model and SAS software (v. 9.1, SAS Institute Inc., Cary, N.C.). Means were compared using the Tukey's minimum significant differences at $P = 0.05$.

Results

Spore germination and secondary germ tube formation

Percent spore germination on leaf disks of six bigleaf hydrangea cultivars ranged from 70% to 76% 1 DAI. Effects of host resistance on spore germination were not statistically different ($P = 0.8452$).

Secondary germ tubes were initiated from the primary appressoria and the other parts of conidia (Fig. 2). Initiation of secondary germ tubes was detected on leaf disks of all bigleaf hydrangea cultivars. However, percents of GCSGT 2 DAI were significantly different ($P = 0.0029$) among cultivars. 'Nikko Blue' supported the highest percent of GCSGT and 'Veitchii' and 'Holstein' had significantly lower percents of GCSGT than the other cultivars (Table 1). The percents of GCSGT on 'Forever Pink', 'Lilacina', and 'Madame Emile Mouillere' were intermediate among the six cultivars (Table 1).

Dead infected epidermal cells

Cell walls of some infected epidermal cells were stained heavily with trypan blue (Fig. 3), which indicated that these cells were dying. Significant differences ($P = 0.0021$) in percent necrotic cells (PDIC) were detected among cultivars. The PDIC for 'Veitchii' were significantly greater than the values for the other cultivars, which were not significantly different from each other (Table 1).

Infection efficiency

For all hydrangea cultivars, branched hyphae developed from secondary germ tubes and formed colonies. However, infection efficiency of the fungus was significantly different ($P < 0.0002$) among cultivars. The infection efficiency was the highest for 'Nikko Blue' and 'Forever Pink', and was

Fig. 3. Haustoria (HA) in epidermal cells and heavily stained cell walls (HSCW) 5 days after inoculation with *Erysiphe polygoni*. Leaf disks were stained with trypan blue and cleared with chloral hydrate. Scale bar represents 40 μm.
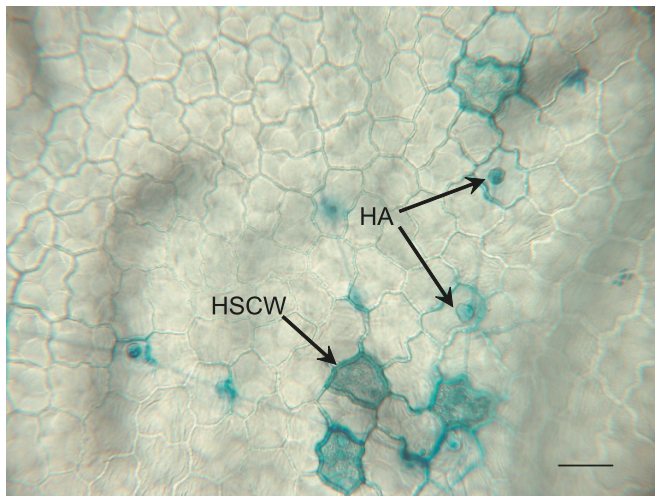
the lowest for 'Veitchii', whereas intermediate infection efficiency was determined for 'Lilacina', 'Madame Emile Mouillere', and 'Holstein' (Table 1).

Latent period

Significant differences (*P* < 0.0001) in latent period of powdery mildew were detected among hydrangea cultivars. The mean latent period for 'Veitchii' was 14 days, and this was significantly delayed compared with the 6-day latent period for all the other cultivars (Table 1).

Sporulation

Compared with the other cultivars, fungal colony development was restricted and sporulation was rare on 'Veitchii' leaf disks (Fig. 4). The effect of cultivars on the variance of spore production was significant (*P* = 0.0001). Sporulation per leaf disk of 'Veitchii' was significantly lower than for the other cultivars. 'Nikko Blue' supported the highest amount of sporulation and was significantly higher than the other cultivars. Production of conidia per leaf disk for 'Forever Pink', 'Lilacina', 'Madame Emile Mouillere', and 'Holstein' was intermediate (Table 1).

Discussion

In the present study, percent spore germination of *E. polygoni* was not significantly different among bigleaf hydrangea cultivars. Spore germination and appressorium formation are necessary for powdery mildew fungi to penetrate the host cuticle and cell wall. Differences in spore germination between susceptible and resistant cultivars have been reported for *Podosphaera leucotricha* (Ell. & Ev.) Salm in apple (*Malus domestica* Borkh.) (Korban and Riemer 1990) and *Erysiphe pisi* DC. in pea (Fondevilla et al. 2007) and barrel medic accessions (Prats et al. 2007). The physical structure of the wax layer on leaf surfaces was reported to stimulate appressorium formation (Ellingboe 1972). This was supported by a recent report that germination of conidia and appressorium differentiation of

Blumeria graminis DC f. sp. *hordei* Marchal were reduced by 20% when leaf cuticle waxes were removed (Zabka et al. 2008). In contrast, spore germination was not different between resistant and susceptible cultivars for *Oidium lycopersicum* Cooke & Massee on *Lycopersicon* species (Huang et al. 1998), *E. pisi* DC on sweet pea (*Pisum sativum* L.) (Poulter et al. 2003; Viljanen-Rollinson et al. 1998), *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci on melon (*Cucumis melo* L.) (Cohen and Eyal 1988; Floris and Alvarez 1996; Kuzuya et al. 2003; Pérez-Garcia et al. 2001), *E. polygoni* DC on mungbean (*Vigna radiate* (L.) Wilczek) (Reddy et al. 2001), and *Erysiphe pulchra* on flowering dogwood (*Cornus florida* L.) (Li et al. 2006). The results of the present study indicate that resistance mechanisms in bigleaf hydrangea were not involved prior to the penetration attempt of the fungus on host leaf surfaces.

Compared with the susceptible 'Nikko Blue', percents of GCSGT was inhibited on the other cultivars with a >43% reduction on 'Holstein' and 'Veitchii' in the present study. Significant reductions of percent secondary germ tube initiation in resistant cultivars indicate that preventing the establishment of a parasitic relationship is one of the important consequences of resistance responses in bigleaf hydrangeas. The fungus must cope with cell-wall-associated defense of the host during penetration. Physical reinforcement of cell walls through the formation of papillae at penetration sites is widely recognized as an early response to microbial attack in host plants (Glazebrook 2005; Jacobs et al. 2003; Li et al. 2007, Nishimura et al. 2003; Skalamera and Heath 1996). Therefore, only a certain portion of germinated spores succeed in penetration, formation of haustoria, and establishment of a biotrophic relationship. In the hydrangea–powdery mildew pathosystem, initiation of secondary germ tubes is a sign of the successful penetration with formation of functional haustoria in epidermal cells and establishment of a parasitic relationship (Li et al. 2009). Strong inhibition of secondary hyphae initiation from appressoria of *Microsphaera alphitoides* was also reported on resistant oak species (Edward and Ayres 1981). In a previous study (Li et al. 2009), we reported that accumulation of callose, a major compound of papillae, under the primary appressoria of *E. polygoni* occurred two days earlier in the resistant cultivar 'Veitchii' as compared with the susceptible cultivar 'Nikko Blue' (Li et al. 2009). Therefore, earlier callose accumulation or papillae formation under penetration sites in the cultivar 'Veitchii' could be a cause of reduced secondary germ tube formation in resistant bigleaf hydrangeas.

Cell necrosis was detected on all six bigleaf hydrangea cultivars regardless of differences in resistance levels. However, the value of PDIC for resistant cultivar 'Veitchii' was significantly higher than for the other cultivars (Table 1). In the hydrangea–powdery mildew pathosystem, haustoria formed under primary appressoria and secondary germ tubes as early as 1 DAI, and necrotic cells were detected in resistant cultivar 3 DAI and susceptible cultivar 5 DAI, respectively (Li et al. 2009). Cell death, known as a sign of HR, is one of the major resistance mechanisms in powdery mildew diseases when the papilla barrier is overcome and the parasitic relationship is established (Tosa and Shishiyama 1984; Johnson et al. 1982). HR is referred to as prehaustorial resistance (Niks and Rubiales 2002) and is

Fig. 4. Fungal colonies and sporulation of powdery mildew on leaf disks of six bigleaf hydrangea cultivars 14 days after inoculation. (A) 'Nikko Blue', (B) 'Forever Pink', (C) 'Lilacina', (D) 'Madame Emile Mouillere', (E) 'Holstein', and (F) 'Veitchii'.
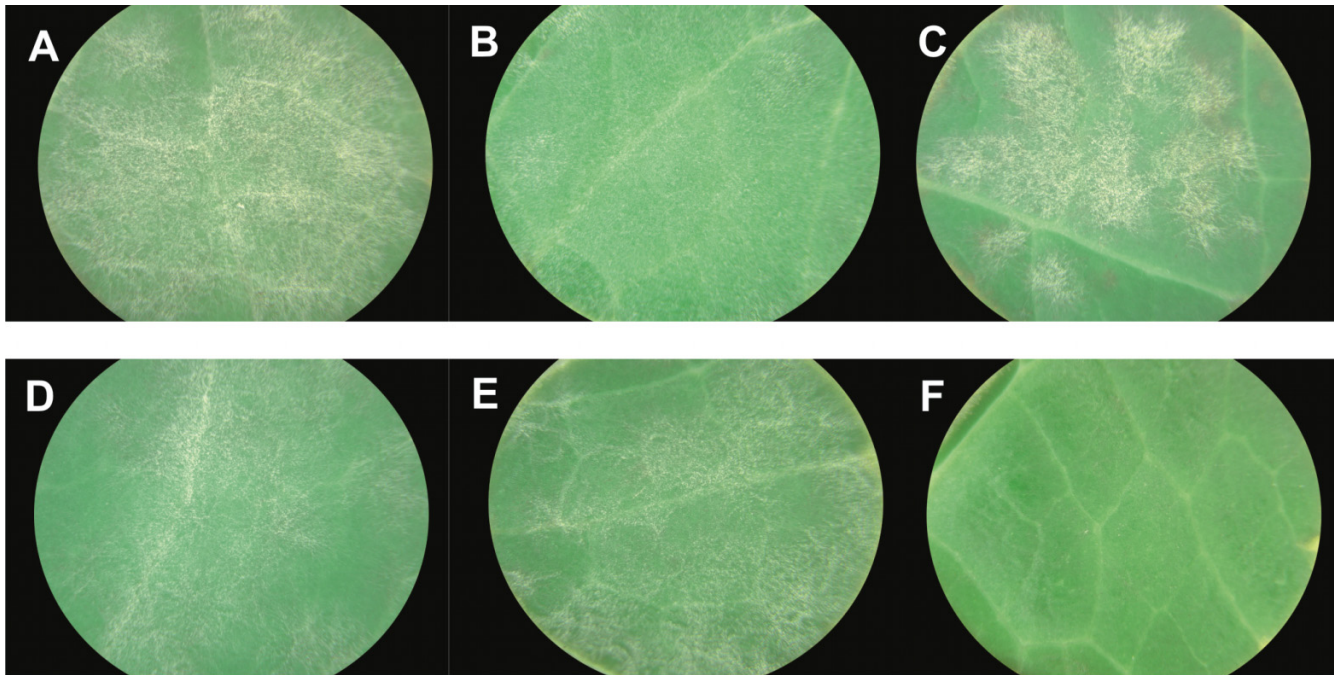
controlled by a single gene in the wheat–rust pathosystems (Ellingboe 1972). HR in a resistant oak species, *Quercus cerris*, almost completely inhibited the formation of the secondary hyphae of *M. alphitoides* (Edward and Ayres 1981). The results of present study suggested that HR was not a qualitative resistance mechanism that completely prevents fungal penetration and haustorium formation in bigleaf hydrangeas, but the quantitative increase of necrotic cells in 'Veitchii' may have made a significant contribution to the resistance components that restrict fungal growth and reproduction.

In the present study, infection efficiency was decreased, latent period was increased, and sporulation was reduced on resistant cultivars although the powdery mildew fungus developed conidiophores and conidia on all bigleaf hydrangeas. The resistance levels of these six bigleaf hydrangea cultivars categorized by using a leaf disk evaluation in the present study mimicked the results of the field observation (Dirr 2004), except the cultivar 'Holstein', which was evaluated as susceptible by Dirr (2004), was grouped as intermediate based on significant less sporulation, lower infection efficiency, and lower percent GCSGT compared with the susceptible cultivar 'Nikko Blue'. The cultivar 'Veitchii' was reported as resistant without symptoms, but not immune (Dirr 2004). Differences in fungal development among cultivars after the establishment of a parasitic relationship with the host suggest that post-haustorial resistance mechanisms exist in bigleaf hydrangeas. Infection efficiency, latent period, and sporulation were considered major epidemiological components and any one or a combination of these could be used to assess host partial resistance (Parlevliet 1989, 1992). However, some components might more adequately represent the partial resistance than others in fields (Viljanen-Rollinson et al. 1998). Latent period was

better correlated with partial resistance in the field than was infection efficiency in the barley-leaf rust system (Parlevliet 1979). In contrast, infection efficiency was more related to field resistance for *B. graminis* f.sp. *tritici* in wheat (Kinane and Jones 2000). Reduction of infection efficiency levels and lower sporulation rates were more important than latent period for the resistance to *E. pisi* in peas (Viljanen-Rollinson et al. 1998). Resistance to *O. lycopersicum* in wild tomato species was also characterized by a very low infection frequency and lack of sporulation (Lindhout et al. 1994). Compared with highly susceptible cultivars, longer latent period and lower sporulation rates of *S. fuliginea* were detected on moderately resistant cultivars of melon (Floris and Alvarez 1996). In the present study, latent periods were not significantly different among cultivars except for the highly resistant 'Veitchii', and this suggests that variables in infection efficiency and sporulation could be used to evaluate bigleaf hydrangeas for resistance to powdery mildew.

Our study demonstrated that resistance to powdery mildew in bigleaf hydrangeas appeared as reduced percent secondary germ tube formulation, increased necrotic cells, decreased infection efficiency, longer latent period, and reduced sporulation, which suggests that resistance of bigleaf hydrangeas to powdery mildew is quantitative or partial resistance (Parlevliet 1992). These resistance components could be used to quantitatively assess bigleaf hydrangeas for powdery mildew resistance using a detached leaf bioassay. Host cell necrosis, although it could not completely stop fungal growth and reproduction of *E. polygoni*, was involved in the resistance to powdery mildew in hydrangeas. The resistance mechanisms of cell necrosis contributing to restricted fungal development and reduced spore production need to be further investigated.

Acknowledgements

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