HRI's Mission:

To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

13. Townsend, A.M. and W.O. Masters. 1984. 'Pioneer' elm. HortScience 19:900.

14. Townsend, A.M., L.R. Schreiber, W.O. Masters, and S.E. Bentz. 1991. 'Prospector' elm. HortScience 26:81–82.

15. Townsend, A.M., L.R. Schreiber, W.O. Masters, and S.E. Bentz. 1991. 'Frontier' elm. HortScience 26:80–81.

16. Townsend, A.M. and F.S. Santamour, Jr. 1993. Progress in the development of disease-resistant elms, p. 46–50. *In*: Sticklen, M.B., and J.L. Sherald (editors). Dutch Elm Disease Research: Cellular and Molecular Approaches. Springer-Verlag. New York, NY.

17. Townsend, A.M., R.W. Hall, and W.O. Masters. 1995. 'Patriot' elm. J. Environ. Hort. 13:113–115.

18. Townsend, A.M., S.E. Bentz, and G.R. Johnson. 1995. Variation in response of selected American elm clones to *Ophiostoma ulmi*. J. Environ. Hort. 13:126–128.

19. Wright, J.W. 1976. Introduction to Forest Genetics. Academic Press. New York. 463 p.

Differential Resistance to Entomosporium Leafspot Disease and Hydrogen Cyanide Potential in Photinia[1]

K. A. Jacobs[2], F. S. Santamour, Jr.[3], G. R. Johnson[4], and M. A. Dirr[5]
The Morton Arboretum, Route 53, Lisle, IL 60546

Abstract

Photinia serrulata Lindl., *P. glabra* (Thunb. ex J.A. Murr.) Maxim., and two of their progeny, *P.* x *fraseri* Dress. 'Birmingham' and 'Kentucky' were screened for susceptibility to Entomosporium leafspot and for hydrogen cyanide potential using detached leaf assays. *Photinia serrulata* was least susceptible to the fungus, while *P.* x *fraseri* 'Birmingham' and *P. glabra* were most susceptible. Young leaves were much more susceptible than mature leaves in all taxa except *P. glabra*, which was found to be acyanogenic. Although distinct differences in hydrogen cyanide potential occurred among the clones, a direct correlation to disease susceptibility was not indicated.

Index words: *Entomosporium mespili*, *Photinia serrulata*, *P. serratifolia*, *P.* x *fraseri* 'Birmingham'. *P.* x *fraseri* 'Kentucky', *P. glabra*, disease screening, detached leaf assay, cyanogenesis.

Significance to the Nursery Industry

Photinia taxa and specifically *P.* x *fraseri* 'Birmingham' are landscape staples in the southern United States, Japan, Australia and New Zealand. However, Entomoporium leafspot disease has significantly reduced the use of the plants. Nursery production of 'Birmingham' has decreased over 50% in the southeastern U.S. during the past six years. The results of this paper show that a high level of resistance occurs within *P. serrulata*. Interclonal variation in resistance was evident between *P.* x *fraseri* 'Birmingham' and 'Kentucky'. The opportunity exists to reconstruct the crosses between *P. serrulata* and *P. glabra* or a more resistant clone of *P.* x *fraseri*, select for vivid red foliage, screen for disease resistance, and back-cross superior seedlings with *P. serrulata* to attain increased disease resistance. Perhaps a multiclonal series with similar ornamental traits and high degrees of leafspot resistance could be introduced. Results of the detached leaf bioassay reported herein correspond with the observed landscape disease evaluations suggesting that the leaf bioassy could be used to rapidly screen seedling populations.

Introduction

Entomosporium mespili (DC. ex Duby) Sacc. (teleomorph = *Diplocarpon maculatum* (Atk.) Jorstad) causes a leafspot disease of 14 genera in the subfamily Pomoideae of the Rosaceae (7). The disease was first observed on *Photinia* spp., both *P. serrulata* Lindl. and *P. glabra* (Thunb. ex J. A. Murr.) Maxim., in Louisiana in 1957 (15). The disease continues to affect *Photinia* taxa grown in nurseries and as landscape plants in the United States, Japan and New Zealand (7).

Resistance to some leafspot diseases are associated with cyanogenesis, in which cleaving of cyanide-containing glucosides in the host, via pathogen and/or host glucosidases, yield hydrogen cyanide (HCN). Infection rates in the copperspot disease of trefoil (*Lotus corniculatus* L.), caused by *Stemphyllium loti* Graham are reduced as leaf HCN levels increase (12). Helminthosporium leafspot and Gloeocercospora leafspot of sudangrass (*Sorghum sudanese* Stapf. 'Piper') and a sorghum hybrid (*Sorghum bicolor* (L.)

[1]Received for publication March 11, 1996; in revised form May 31, 1996. This project was partially funded by a grant from **The Horticultural Research Institute, 1250 I Street, N.W., Suite 500, Washington, DC 50005**. We thank Ruth Dix for collecting inoculum and assisting in disease screening assays; Louise Riedel for assistance in HCN assays, and John C. Knowlton, III, Lázló Csiba, and Johann Klodzen for additional technical assistance. We appreciate the review comments made by Drs. Gary Watson and Charles Wilson.

[2]Research Plant Pathologist, The Morton Arboretum, Route 53, Lisle, IL 60532.

[3]Research Horticulturist, Floral and Nursery Plants Research Unit, U.S. National Arboretum, 3501 New York Ave., NE, Washington, DC 20002.

[4]Research Plant Geneticist, U.S.D.A. Forest Service, PNW Research Station, Corvallis, OR 97330.

[5]Professor, University of Georgia, Dept. of Horticulture, Athens, GA 30602.

Moench. x sorghum hybrid) are also reduced with increasing HCN evolution from leaves (13).

Gibbs (3) reported that leaves of *P. serrulata* and several other species were cyanogenic, and Hegnauer (6) stated that the cyanogenic glycoside in *Photinia* leaves was prunasin. A preliminary survey of cyanogenesis in *Photinia* was made in March, 1995 at the U.S. National Arboretum, and we found that a clone of *P. glabra* was virtually acyanogenic, while *P. serrulata* 'Green Giant' produced more than twice as much HCN as two *P.* x *fraseri* W. J. Dress. cultivars. *Photinia* x *fraseri* cultivars are progeny of crosses between *P. serrulata* and *P. glabra*.

To our knowledge, no reports of variation to Entomosporium leafspot have been reported within *Photinia*. Our objectives were to evaluate and compare resistance to the disease in clones of *P. serrulata*, *P. glabra*, *P.* x *fraseri* 'Birmingham', and *P.* x *fraseri* 'Kentucky', and determine if leaf HCN levels were related to disease development.

In a recent publication from the U.S. National Arboretum, Meyer et al. (11) listed *P. serratifolia* (Desf.) Kalk. as the valid name for *P. serrulata* Lindl. This change was based on a listing in Index Kewensis (Suppl. XVI, 1981, p. 212) that considered the change proposed by Kalkman (9) as being taxonomically well-founded. The adoption of this new nomenclature has been erratic (2, 8), and until such time as there is a consensus for the name change, the continued use of *P. serrulata* appears reasonable.

Materials and Methods

Plant material. Five plants of individual clones of *P. glabra*, *P. serrulata* 'Green Giant', *P.* x *fraseri* 'Birmingham', and *P.* x *fraseri* 'Kentucky' were used as leaf sources for the detached leaf and HCN assays. The plants were grown in containers for two years, and were maintained in a greenhouse for eight months prior to inoculation to avoid natural infection by the pathogen.

Inoculum preparation. Entomosporium mespili spores were collected in the Winter of 1994–95 from naturally infected, *P.* x *fraseri* 'Birmingham' growing in a commercial parking lot in the greater Washington, DC, area. Symptomatic leaves were collected as needed to provide a continual supply of inoculum. Leaves were incubated in darkness immediately after collection at 20C (68F) and 92% humidity for two weeks, to help induce sporulation. Erratic sporulation necessitated that large numbers of leaves (e.g. 200) be used to obtain a 500 ml spore suspension at a concentration of 3×10^4 spores ml^{-1}. The suspension was prepared on the day of the assay by scraping leaf surfaces in sterile, distilled water.

Detached leaf assay. Four 'young' leaves from the current year's growth (1995), and four mature leaves from the previous year's growth (1994) were selected from each plant. The young leaves were fully expanded but still succulent, and they were lighter green than the mature leaves. Detached leaves were immediately wrapped in a moist paper towel and placed in a cooler. Leaf surface area was measured with an area meter (LI-Cor LI3100). Each leaf was dipped into the spore suspension so that both abaxial and adaxial surfaces were coated, and the petiole of each leaf was then placed through a hole in a styrofoam square. The styrofoam was floated on water in a 60 cm (24 in) × 37.5 cm (15 in) × 10 cm (4 in) container (Fig. 1), ensuring that all petioles were in contact with water. A young and mature leaf from each plant was placed in each of four containers, so that a total of 40 leaves were in each container. One container was used for the control to check for natural infection, and those leaves were dipped in water. All containers were covered loosely with plastic wrap to maintain high humidity, and placed in a growth chamber at 20C (68F) and 92% humidity. After 24 hr of darkness to encourage infection, the growth chamber was set for a 14 hr daylength. A total of 8 leaves per plant (160 leaves per clone) were assayed.

Disease assessment. The number of lesions formed on each leaf was counted at 7, 10, 14, and 21 days, and the percentage of total leaf surface that was necrotic was visually estimated at 21 days. Measurements were calculated per cm^2 of leaf surface. The data were analyzed for differences among and between taxa, and between young and mature leaves using the ANOVA procedure (14).

Hydrogen cyanide assessment. Cyanogenesis from leaf tissue was determined using the well-known sodium picrate test with a few refinements. The alkaline picrate solution was prepared by dissolving 25 g sodium carbonate and 5 g picric acid in 1 liter of distilled water. Strips of 3 MM filter

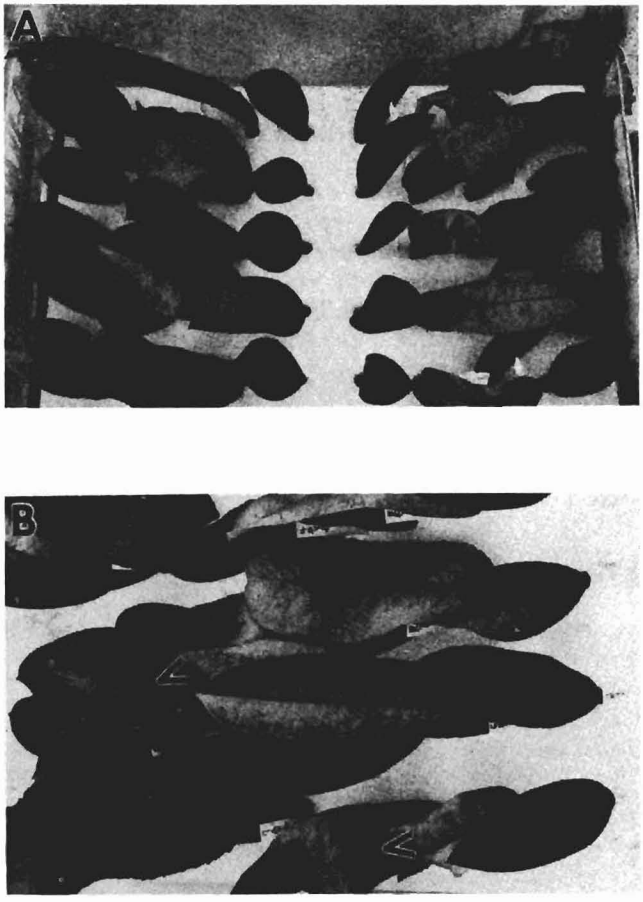

Fig 1. A). Photograph of container used in disease screening assay. Petioles extended through pre-formed holes in styrofoam that was floated on water. B). Close-up of leaves after incubation. Note spotting symptom (arrowheads).

paper (Whatman) (10 cm × 1 cm) were dipped into this solution to a depth of 4 cm, and allowed to dry at room temperature. Strips that showed exactly a 5 cm level of sodium picrate were used to develop a standard curve based on recovery of HCN from known concentrations of KCN (4).

Three plants representing each clone were sampled for hydrogen cyanide potential. On three successive days, one new shoot bearing 8–9 young leaves, and 3 randomly selected mature (1994) leaves were harvested from each plant. The leaves were rinsed in distilled water, patted dry, and their areas were determined on an area meter (LI-Cor LI3100). Laminar tissue (no midrib) of each leaf was finely chopped and approximately 0.1 g was weighed out and placed in a 150 mm × 16 mm test tube fitted with a ground glass stopper. Five drops of chloroform were added to the chopped leaves and a filter paper strip, prepared as above, was suspended above the tissue. The paper strips were removed after incubation at 25C (77F) for 24 hr, and the reddish HCN-picrate complex was eluted in 7 ml distilled water for 30 min. The absorbtion of the eluate at 515 nm was determined with a spectrophotometer (Bausch and Lomb "Spectronic 20"). A fresh paper strip was placed in each tube for a second 24-hr period, and HCN levels were determined using the standard curve, adjusting for the fresh weight of each sample. Total HCN content, expressed as µg/g dry weight of tissue, was calculated for a total of 102 young leaves (25–26 per clone), and 36 mature leaves (9 per clone).

Results and Discussion

Photinia serrulata 'Green Giant' developed the least number of lesions and necrosis of the taxa tested, while *P.* x*fraseri* 'Birmingham' generally developed the most (Table 1). Twenty-one days after inoculation, young 'Green Giant' leaves had an average of 0.13 lesions/cm^2 leaf tissue, compared to 1.49 lesions/cm^2 in 'Birmingham'. *Photinia glabra* and 'Kentucky' had intermediate numbers of lesions (0.23 and 0.68, respectively). Similarly, young 'Green Giant' leaves were least necrotic (2.2%), and 'Birmingham' young leaves were most necrotic (22.7%). The ranking of taxon susceptibility was similar in mature and young leaves, except that mature leaves of *P. glabra* appeared to be equally, if not more susceptible to leafspot than 'Birmingham'. In a preliminary study comparing detached leaves from three plants per clone, *P. glabra* appeared most susceptible as it developed three times as many lesions as the hybrids, and five times as many lesions as 'Green Giant'. *Photinia serrulata* exhibited nearly complete resistance to leafspot in that study. Our findings correspond well to field observations in the southeastern United States in which *P. serrulata* 'Green Giant' appears resistant to leafspot, while both 'Birmingham' and *P. glabra* are susceptible (M. A. Dirr, pers. comm.).

Symptom severity depended on leaf age in all taxa except *P. glabra*. The number of lesions in young leaves of 'Green Giant' and the *P.* x*fraseri* cultivars was 10 to 30 times greater than in mature leaves, and the percentage of leaf necrosis was roughly 10-fold greater than in mature leaves. Baudoin (1) found that young, expanding leaves of *P.* x *fraseri* were much more susceptible to Entomosporium leafspot than mature leaves, and he attributed this to the thicker cuticle in mature leaves.

Both young and mature leaves of *P. glabra* were acyanogenic, although they contained an enzyme capable of cleaving prunasin. In contrast, *P. serrulata* 'Green Giant' and the two *P.* x *fraseri* cultivars tested positive for HCN (Table 1). There was considerable intra-clonal variation in HCN levels (note ranges in Table 1), but the average HCN content in young leaves of 'Green Giant' was 1652 µg/g, more than three times as much as the combined average (541 µg/g) for 'Birmingham' and 'Kentucky'. The average HCN level in mature leaves of 'Green Giant' was much less than that found in young leaves, but was still more than twice the combined average of the *P.* x *fraseri* clones (316 µg/g).

The data are inconsistent in defining the relationship, if any between HCN content and leafspot resistance. Some of the results support the idea that HCN might have enhanced leafspot development. For example, young leaves generally had higher levels of HCN than mature leaves, and young leaves developed more symptoms than mature leaves. Also, the acyanogenic *P. glabra* was the only clone in which mature leaves were as susceptible to leafspot as young leaves. Leaf HCN is known to enhance growth of some fungi, for example, the leaf blight pathogen *Microcyclus ulei* (P. Henn.) V. Arx in the rubber tree (*Hevea brasiliensis* Müell. Arg.) (10).

Other data suggest that HCN might have offered some protection against disease. *Photinia serrulata* had the highest levels of HCN and developed the fewest symptoms. The lack of a clear relationship between HCN and resistance

Table 1. Leafspot symptoms caused by *Entomosporium mespili* and hydrogen cyanide content of leaves in four *Photinia* taxa.

Taxon	Necrosis (%) mature lvs[z]	Necrosis (%) young lvs[z]	Lesions (#/cm^2) mature lvs[y]	Lesions (#/cm^2) young lvs[y]	HCN (µg/g d.w.) mature lvs[x]	HCN (µg/g d.w.) young lvs[w]
P. serrulata 'Green Giant'	0.2a[v]	2.2a	0.00a	0.13a	751 (435–875)	1652 (1290–1848)
P. x*fraseri* 'Birmingham'	2.6c	22.7b	0.08a	1.49b	437 (219–511)	485 (226– 650)
P. x*fraseri* 'Kentucky'	1.4b	8.8a	0.07a	0.68ab	195 (138–352)	597 (377– 812)
P. glabra	2.0c	4.3a	0.23b	0.23a	acyanogenic	acyanogenic

[z]Values represent mean percentage of leaf necrosis 21 da after inoculation for 4 mature leaves and 4 young leaves from each of 5 plants per clone.

[y]Values represent the total number of lesions 21 da after inoculation for 4 mature leaves and 4 young leaves from each of 5 plants per clone.

[x]Values represent means and ranges of 9 mature leaves on each of 3 plants per clone.

[w]Values represent means and ranges of 25–26 young leaves on each of 3 plants per clone. A significant ($p = 0.05$), positive correlation existed between HCN content and leaf size in each clone.

[v]Lowercase letters indicate significant differences at the $p = 0.05$ level according to Tukey-Kramer's test of means separation.

might be due to a lack in sensitivity of *E. mespili* to HCN. If a fungus is able to detoxify HCN, as is the case with numerous pathogens of cyanogenic plants (5), disease development will not have a direct relationship to the total amount of HCN (13). It would be necessary to examine the sensitivity of *E. mespili* to HCN in order to clarify the relationship between cyanogenesis in *Photinia* and leafspot resistance.

The disease severity and HCN data indicate potentially meaningful differences between *Photinia* taxa. *Photinia serrulata* 'Green Giant' appeared to be least susceptible to leafspot, although the amount of disease was not always significantly lower than in other taxa. *Photinia* x *fraseri* 'Birmingham' was more susceptible than the other taxa overall, but older foliage of *P. glabra* was also highly susceptible. This is important given the widespread use of 'Birmingham' in the landscape industry.

It may be possible to use the detached leaf inoculation assay to select resistant, yet attractive 'red-tipped' plants from the progenies of controlled backcrosses between *P. serrulata* and *P.* x *fraseri*. The assay was quick, and should be reproducible as long as leaf age is noted. The limiting factor in the assay was the difficulty in obtaining inoculum, and given the fact that *E. mespili* grows poorly on artificial media, this will not be an easy problem to solve. If a clearer relationship between cyanogenesis and leafspot resistance can be defined, backcross progenies might also be screened for HCN potential as an indicator of resistance.

Literature Cited

1. Baudoin, A.B.A.M. 1986. Infection of photinia leaves by *Entomosporium mespili*. Plant Dis. 70:191–194.

2. Brickell, C. (Ed.) 1989. Gardener's Encyclopedia of Plants and Flowers. Royal Hort. Soc., Dorling Kindersley, London. p. 538.

3. Gibbs, R.D. 1974. Chemotaxonomy of Flowering Plants. Vol. 3. McGill-Queens Univ. Press, Montreal, Canada. pp. 1621–1622.

4. Gilchrist, D.G., W.E. Lueschen, and C.N. Hittle. 1967. Revised method for the preparation of standards in the sodium picrate assay of HCN. Crop Sci. 7:267–268.

5. Fry, W.E. and P.H. Evans. 1977. Association of formamide hydro-lyase with fungal pathogenicity to cyanogenic plants. Phytopathology 67:1001–1006.

6. Hegnauer, R. 1973. Chemotaxonomie der Pflanzen. Vol. 6., p. 119. Birkhauser Verlag, Basel and Stuttgart.

7. Horie, H. and T. Kobayashi. 1980. Entomosporium leaf spot of Pomoideae (Rosaceae) in Japan. III: Additional basis for identification of the fungus, and distribution of the disease. Eur. J. For. Path. 10:225–235.

8. Huxley, A. (Ed.) 1992. The New Royal Horticultural Society Dictionary of Gardening. Vol. 3. Macmillan, London. p. 558.

9. Kalkman, C. 1973. Malesian species of the subfamily Maloideae (Rosaceae). Blumea 21:413–442.

10. Lieberei, R. 1988. Relationship of cyanogenic capacity (HCN-c) of the rubber tree *Hevea brasiliensis* to susceptibility to *Microcyclus ulei*, the agent causing South American leaf blight. J. Phytopathology 122:54–67.

11. Meyer, F.G., P.M. Mazzeo, and D.H. Voss. 1994. A Catalog of Cultivated Woody Plants of the Southeastern United States. U.S. National Arboretum Contribution No. 7. Superintendent of Documents, Washington, DC.

12. Millar, R.L. and V.J. Higgins. Association of cyanide with infection of birdsfoot trefoil by *Stemphyllium loti*. Phytopathology 60:104–110.

13. Myers, D.F. and W.E. Fry. 1978. Hydrogen cyanide potential during pathogenesis of sorghum by *Gloeocercospora sorghi* or *Helminthosporium sorghicola*. Phytopathology 68:1037–1041.

14. SAS Institute, Inc. 1994. SAS/STAT User's Guide (vers. 6, 4th ed.). SAS Institute, Inc., Cary, NC.

15. Stathis, P.D. and A.G. Plakidas. 1959. Entomosporium leaf spot of *Photinia glabra* and *Photinia serrulata*. Phytopathology 49:361–365.