HortScience 41(1):270–271. 2006.

Leaf Disk Infection by *Colletotrichum acutatum* and Its Relation to Fruit Rot in Diverse Blueberry Germplasm

Mark K. Ehlenfeldt[1], James J. Polashock, and Allan W. Stretch
U.S. Department of Agriculture, Agricultural Research Service, Fruit Laboratory, Henry A. Wallace Agricultural Research Center, Beltsville, MD 20705

Matthew Kramer
U.S. Department of Agriculture, Agricultural Research Service, Biometrical Consulting Service, Henry A. Wallace Agricultural Research Center, Beltsville, MD 20705

Additional index words. image analysis, anthracnose, *Vaccinium*, *Colletotrichum gloeosporioides*

Abstract. **Response to foliar infection by *Colletotrichum acutatum* Simmonds ex Simmonds was assayed in a diverse group of 149 blueberry cultivars and selections using a detached leaf-disk assay. Disks were inoculated and incubated for one week, then were digitally imaged, and images analyzed for percent leaf decay. Infection percentages across cultivars averaged 32%, and ranged from 8% to 79%. The lowest levels of foliar infection were seen in the cultivars, Burlington, Sharpblue, and Berkeley. Foliar responses were compared to anthracnose fruit rot susceptibility data from a previous study. Several clones were observed to have low levels of both foliar and fruit infection. Cultivars with particularly good resistance to both phases included 'Sharpblue', 'Sunshine Blue', 'Legacy', 'Little Giant', 'Flordablue', 'Elliott', 'Blue Ridge', 'Blue Rose', and 'November Glow'. Little correlation was observed between foliar response and fruit response to anthracnose infection ($r = 0.15$). Since *C. acutatum* overwinters primarily in vegetative tissue, breeding new cultivars with foliar resistance may assist in reducing inoculum levels of this disease under field conditions.**

Anthracnose fruit rot, caused by *Colletotrichum acutatum,* affects commercially important highbush (Stretch, 1967), rabbiteye (Smith et al., 1996), and lowbush (Lambert, 1990) blueberries. The disease occurs in most of the important blueberry growing regions in the U.S. and British Columbia (Daykin and Milholland, 1984; Hartung et al., 1981; Smith et al., 1996; Stretch, 1967; Verma et al., 2003). During infection, conidia germinate on the surface of green fruit and form appressoria. The hyphae then penetrate the epidermis, but remain latent until the fruit begin to ripen (Daykin and Milholland, 1984). Rot symptoms ultimately appear as visible orange-colored spore masses on the fruit surface (Milholland, 1995). Although anthracnose is generally considered to be a problem on fruit, the pathogen can also infect leaves (Milholland, 1995). Infections in vegetative tissues (Milholland, 1995) and dormant flower buds serve as primary sources of inoculum for the following year (DeMarsay and Oudemans, 2002). Although the causal agent of anthracnose fruit rot in some growing areas is reported as *C. gloeosporioides*, only *C. acutatum* isolates have been identified in New Jersey in collections made since 1994 (P. V. Oudemans, personal communication), and our report is limited to this species.

A primary objective of the U.S. Department of Agriculture blueberry breeding program has been to identify sources of fungal disease resistance with the ultimate goal of reducing fungicide applications. As part of this program, a collection of highbush blueberry cultivars has been screened for resistance to mummy berry blight (Ehlenfeldt et al., 1996), mummy berry fruit rot (Stretch and Ehlenfeldt, 2000), and anthracnose fruit rot (Polashock et al., 2005). Because of the need for plants bearing fruit, and the need for postharvest incubation to fully manifest symptoms, anthracnose fruit rot resistance screening in a nursery-type setting is time consuming, as well as material and labor intensive. An assay that could use nonflowering plants or seedlings would be extremely useful when selecting for resistance to this disease. In addition, foliar resistance may reduce overwintering inoculum. The objective of this study was to evaluate quantitative differences in foliar responses to anthracnose infection in blueberry cultivars, and to determine whether these responses were correlated to known fruit-infection responses.

Materials and Methods

Cultivars and inoculations. A group of 149 cultivars and selections representing many commonly grown types of blueberries was selected for screening. These cultivars included 71 northern highbush (*Vaccinium corymbosum* L.), 26 southern highbush (*V. corymbosum*, usually with some introgressed *V. darrowi* Camp and/or *V. ashei* Reade), 36 rabbiteye (*V. ashei*), 5 lowbush (*V. angustifolium* Ait.), 7 'half-high' (*V. corymbosum* × *V. angustifolium* derivatives), and 4 mixed-species derivatives. A listing of the cultivars and their pedigrees is available from the authors. Leaves were sampled from in-ground demonstration plots at the P.E. Marucci Center for Blueberry and Cranberry Research, Chatsworth, N.J. Mature leaves were collected from plants on 5 and 9 Sept. 1997 and on 5 and 12 Aug. 1998. In each year, two trials (blocks) were completed, with the trials being initiated the same days that the leaves were collected. For each trial, leaf disks were excised from six leaves of each clone using a 10-mm cork borer. Each replication within a trial consisted of a set of 149 disks, representing all of the genotypes, placed within a plastic container [32 cm (L) × 23 cm (W) × 10 cm (H)] on moistened paper towels. Each trial consisted of five inoculated replicates (sets) and one control replicate.

Colletotrichum acutatum isolates were grown on solid V8 juice agar and V8 juice liquid medium as previously described by Polashock et al. (2005). Conidia were diluted in water (with 2 drops of Tween 20 wetting agent/L) to a final concentration of 1×10^6 spores/mL. In 1997, the *C. acutatum* isolate was collected from infected berries of 'Bluecrop' on a commercial farm in Atlantic County, N.J. In 1998, a single-spore culture was isolated from infected berries collected on a commercial farm in Burlington County, N.J.

Disks were inoculated with the *C. acutatum* spore solution by spraying each box to saturation (90 mL of inoculum was used across 5 boxes). A control inoculation on a single set of disks was made following the same procedure using only water with the wetting agent.

After a 1-week incubation at room temperature (about 20 °C), disks were digitally imaged, with an IS-500 gel documentation system (black and white) (Alpha Innotech Corp., San Leandro, Calif.) using a video camera fitted with a Green 1 filter (Tiffen Corp., Hauppage, N.Y.) to enhance contrast between healthy and decayed tissue. Images were saved as 30 pixel/cm TIFF-files (area of each disk = about 1000 pixels) and analyzed using Optimas, Version 6.1 image analysis program (Media Cybernetics, Inc., Silver Spring, Md.) to determine the percent infected (i.e., necrotic) area. Images in Optimas were coded initially as values within a range of 256 levels of gray, with 0 = pure black and 255 = pure white. Contrast was adjusted as needed to accurately delimit the necrotic areas. Delineation lines were automatically generated in Optimas using a brightness threshold set manually that was based on visual observation of necrotic tissue. In cases where contrast between healthy and infected tissue was poor, lines were drawn manually based on direct observation of the disk in question.

Statistical analysis. Data were transformed from the proportion scale for analysis using z = arcsine (square root (p)), where p represents

Received for publication 20 Sept. 2005. Accepted for publication 3 Nov. 2005. The authors thank Robert Martin for his dedication in performing extensive image analysis measurements and for other technical assistance.

[1]To whom reprint requests should be addressed: P.E. Marucci Center for Blueberry and Cranberry Research, 125A Lake Oswego Road, Chatsworth, NJ 08019; e-mail mehlenfeldt@ars.usda.gov.

the proportion of necrotic area of the leaf disk, to satisfy the ANOVA assumption of homogeneous variances. A preliminary analysis suggested that there was no overall year effect, and the cultivar × year interaction effect was large for only a very few cultivars (as would be expected by chance when there are a large number of cultivars in the data set), so these terms were not included in our modeling. Some year-to-year and trial-to-trial variation was captured by including a year × trial random variable (4 levels) in a mixed model, with cultivar as the only fixed effect (149 levels). The model, estimated using Proc Mixed (SAS Institute, Cary, N.C.), produced means and standard errors (SE) for each cultivar. The values were back-transformed to the original (proportion) scale along with the mean ±2 SE, yielding a 95% confidence interval for each cultivar. For the 95 cultivars common to this study and the one by Polashock et al. (2005), the correlation between the anthracnose fruit rot and necrotic leaf disk area was calculated, and cultivars showing resistance to both modes of infection were identified.

Results

Portions of most leaf disks were necrotic after the 1-week incubation. When incubated for longer periods of time, all leaf disks reached 100% infection. Analysis of variance showed significant differences among cultivars ($F = 7.13$, $P < 0.0001$). Leaf infection percentages across cultivars averaged 32% and ranged from 8% to 79%. In general, noninoculated controls showed low levels of necrosis, typically not >1% to 2%. Because of the large number of cultivars used, the means showed an almost continuous gradation from relatively susceptible to relatively resistant. Varieties with lower relative foliar infection levels included the northern highbush cultivars 'Burlington', 'Berkeley', 'Katharine', and 'Reka', and the southern highbush cultivar 'Sharpblue'. Those cultivars that showed high relative foliar infection levels included the highbush cultivar 'Puru', the lowbush cultivar 'Brunswick', and the half-high cultivars 'Friendship', 'St. Cloud', and 'Chippewa'. A complete list of cultivars and their response values may be obtained from the authors. Highbush, southern highbush, and rabbiteye were, by and large, distributed across the total range of disease expression. However, the half-high cultivars (50% lowbush germplasm), as a group, tended to have higher levels of foliar infection, and all seven were in the highest third of the distribution. The probability of such a distribution occurring by chance versus a random distribution throughout the range; and assuming independence (i.e., that they are genetically distinct) is $(1/3)^7 = 5.65 \times 10^{-6}$. The reason for this commonality of ranking may lie both in the nature of the germplasm (lowbush) and in a certain commonality of specific ancestry in several of the half-high cultivars. Similarly, four of the five lowbush cultivars fell in the upper third of the distribution. Thus, pure lowbush cultivars also appear to be relatively susceptible as a group.

Fruit infection values for cultivars reported in Polashock et al. (2005) were found to have little correlation to foliar responses to infection ($r = 0.15$, $P = 0.15$, t test, 93 df) for the 95 cultivars common to both studies. Only one cultivar, Sharpblue, exhibited very good resistance to both foliar (0.08) and fruit (0.23) infection. Eight additional cultivars exhibited low fruit infection and had leaf infection values below the mean (0.32) (Table 1).

Discussion

There were significant differences in the relative levels of foliar infection by *C. acutatum* with a wide range of infection ratings. There were no cultivars that exhibited absolute resistance, but this was not unexpected since the pathogen was applied to wounded leaf margins. The apparent relative susceptibility of *V. angustifolium* germplasm suggests that anthracnose may be a greater foliar problem in lowbush and lowbush derivatives; however, the determination of how our experimentally derived measures of foliar resistance relate to intact leaves and tissues is unknown, and would be an important further step in extrapolating this information. The observed differences suggest that the leaf-disk assay could be used to screen seedlings for foliar resistance to this pathogen. Unfortunately, the lack of correlation between foliar and fruit rot susceptibility to this pathogen indicates that this assay is not useful as a predictor of susceptibility to fruit rot caused by anthracnose.

Table 1. Cultivars with below average (<0.32) proportions of leaf infection and with low levels of fruit infection.

Cultivar	Type[z]	Avg proportion decayed	
		Leaf disk	Fruit[y]
Sharpblue	SHB	0.08	0.23
Sunshine Blue	SHB	0.21	0.12
Legacy	SHB	0.23	0.10
Little Giant	RE-CON	0.24	0.09
Flordablue	SHB	0.24	0.14
Elliott	HB	0.25	0.15
Blue Ridge	SHB	0.26	0.18
Blue Rose	HB	0.29	0.16
November Glow	HB	0.30	0.14

[z]Germplasm types: SHB = southern highbush, HB = highbush, RE-CON = rabbiteye × *V. constablaei* Gray.
[y]From Polashock et al., 2005.

Colletotrichum acutatum growth on vegetative tissues can be an important source of inoculum for fruit infection of strawberry (Leandro et al., 2001) and citrus (Zulfiqar et al., 1996). Similarly, in blueberry, *C. acutatum* inoculum overwinters primarily in vegetative tissues (Milholland, 1995) or floral buds (DeMarsay and Oudemans, 2002), each of which can serve as a source of inoculum. Foliar resistance to *C. acutatum* infection may result in lower levels of springtime inoculum, and hence foliar resistance may result in lower levels of in-season fruit infection. The information regarding foliar responses may also be useful in making management decisions to control anthracnose (i.e., as a predictor of inoculum levels and thus disease pressure). Current information suggests that *C. acutatum* is observed more commonly on blueberry than is *C. gloeosporioides*, nonetheless, the latter pathogen causes fruit infections in some areas. Since we have not seen *C. gloeosporioides* in New Jersey, we did not extend our assays to this species, but it is possible that different leaf responses may occur with *C. gloeosporioides*.

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