Evaluation of Resistance to *Erwinia amylovora* and *Botryosphaeria dothidea* in Eastern U.S. Blackberry Cultivars

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Summary. In order to gauge the level of resistance available, eleven blackberry cultivars were inoculated with two different pathogens, the bacterium *Erwinia amylovora* and the fungus *Botryosphaeria dothidea.* Primocanes on 1-year-old plants were greenhouse inoculated with *E. amylovora* by injecting a sterile water suspension of the pathogen into the shoot-tips. Ten days after inoculation, these plants were rated for severity of symptoms on a scale of 0 to 5. Primocane cuttings 7 in. (18 cm) in length, taken from mature field plots, were inoculated with *B. dothidea* by placing a 1/8th in. (3 mm) square of mycelia into an incision, and incubating them at high humidity on a lab bench. Ten days following inoculation, the resulting lesions, if any, were measured and the area computed. Both resistant and susceptible cultivars were identified for each of the two diseases. The most resistant cultivars to *E. amylovora* were 'Kiowa', Prime-Jim™ and 'Arapaho', while 'Triple Crown' and 'Arapaho' were the most resistant to *B. dothidea.*

Blackberries (*Rubus* subgenus *Rubus*) are a crop of growing importance in Arkansas and the United States, and the University of Arkansas breeding program is among the largest in world. While blackberries are generally considered a relatively disease-free crop and most blackberry diseases are infrequent problems, some have on occasion caused significant damage. As blackberries continue to be planted in varied climates and in increasing numbers, the possibility exists that currently minor diseases may become serious issues. Prompted by an unexplained outbreak of shoot-tip blight in 'Apache', which was observed in research plots in 2001, inoculation studies were conducted in order to gauge resistance of important cultivars of eastern U.S. blackberries to two cane blight pathogens: the bacterium *Erwinia amylovora* Burr. (Winsl. et al.), and the fungus *Botryosphaeria dothidea* (Moug. Ex Fr.) Ces. & de Not.

Erwinia amylovora causes fire blight, a bacterial disease affecting many Rosaceous species, with apple (*Malus* sp.) and pear (*Pyrus* sp.) being of the greatest economic significance (Bonn and van der Zwet, 2000). Outbreaks in *Rubus* species, while less common, do occur, including incidents on blackberries in eastern Oklahoma (S. von Broembsen, personal communication), and Illinois (Ries and Otterbacher, 1977). Fire blight has been reported on red raspberries (*Rubus idaeus* L.) in Wisconsin (Heimann and Worf, 1985) and Canada (Braun et al., 1999; Evans, 1996), where it has become a significant problem in some years. Although the strain of *E. amylovora* responsible for fire blight in *Rubus* species is generally considered to be different than that responsible for the disease in all other hosts, at least one incident of infection by an apple isolate in raspberry has been reported (Evans, 1996). While Ries and Otterbacher (1977) found several resistant raspberry cultivars, including 'Fall Red' and 'Heritage', none of the nine blackberry cultivars included in their study demonstrated any resistance to the pathogen.

Botryosphaeria dothidea is the causal agent of *Botryosphaeria* cane canker, a fairly uncommon disease of thornless blackberries (Maas and Uecker, 1984). It is a common facultative parasite on dead and dying tissue in many woody species, and a pathogen on several important species, including apple, peach (*Prunus persica* (L.) Batsch), and blueberry (*Vaccinium* sp.), in addition to blackberries. In blackberries, it causes dark, spreading lesions at wound sites on mature tissue, sometimes girdling canes entirely. In severe cases, removal of entire plantings has been necessary (Maas and Uecker, 1984). Maas and Uecker (1984) compared both lengths of lesions and subjective ratings of six cultivars, and found significant differences among genotypes using both measurements. However, little is known about most of the cultivars currently important in the eastern United States.

Materials and methods

Fire Blight Resistance Study: An isolate of *E. amylovora* bacterium taken from affected blackberry plants in eastern Oklahoma was obtained in 2002 from S. von Broembsen at Oklahoma State University, Stillwater, Okla. This was streaked on *Erwinia* selective medium (Crosse and Goodman, 1973) and confirmed as *E. amylovora* by colony morphology, then grown on liquid 523 medium (Kado and Heskett, 1970) and stored in 15% glycerol solution at -20°C. Two days before inoculation, this solution was streaked on plates of 523 medium and incubated at 28°C until immediately before inoculation. Two loopfulls of bacteria were taken from the plate and placed in 10 ml of sterile distilled water and mixed vigorously to provide a uniform liquid suspension.

Dormant root cuttings (~10 cm or 4 in. in length) were taken from mature research plantings at the University of Arkansas Agricultural

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Research and Extension Center, Fayetteville, and the University of Arkansas Fruit Substation, Clarksville, in December 2002. Twelve to 15 cuttings of each cultivar were potted in Sunshine Mix LC1 (SunGro Horticulture Inc., Bellevue, Wash.) potting mix amended with 50 ml of Osmocote Vegetable & Bedding Slow Release Plant Food (The Scotts Company, Marysville, Ohio) fertilizer (14-14-14) per 0.03 m^3 of soil in February 2002, and grown in the greenhouse. Of the resulting plants, five were selected of 'Apache', 'Arapaho', 'Navaho', 'Chester Thornless', 'Triple Crown', 'Illini Hardy', and and 'Prime-Jim'™ for the first trial, and six plants of each these cultivars, as well as 'Kiowa', 'Chickasaw', 'Ouachita', and 'Shawnee', were selected for the second trial. Both trials were conducted according to a randomized complete block design in the greenhouse. Greenhouse temperature was maintained at 24° day /22°C night, and plants were kept humid with misting at 9 AM and 4:30 PM each day. The first trial began 5 May 2003, while the second began 1 June 2003.

Plants were inoculated by inserting a No. 30-gauge hypodermic needle completely through the stem just below the first fully unfolded leaf. Sufficient liquid was ejected to completely fill the wound and leave a droplet on either side (Norelli et al., 1988). Additionally, two plants each of 'Arapaho', 'Apache', and 'Navaho' were injected with sterile water as controls. Ten days following inoculation, plants were rated on a scale of 0 to 5 based on the severity of their reaction, with 0 being no visible symptoms, and 5 being complete necrosis from the point of inoculation to the tip of the shoot. Ratings data were analyzed using t-test and analysis of variance functions of the JMP 5.0 statistical software package (SAS Institute, Cary, N.C).

Botryosphaeria Cane Canker Resistance Study. An isolate of the *B. dothidea* fungus was obtained from a research planting of 'Apache' blackberries in Fayetteville, Arkansas in September of 2001. Cultures were maintained at room temperature on plates of potato dextrose agar (PDA) medium with streptomycin (75 mg/L).

Cuttings of primocanes were taken from 11 cultivars of blackberry: 'Arapaho', 'Apache', 'Navaho', 'Ouachita', 'Chickasaw', 'Kiowa', 'Shawnee', 'Illini Hardy', 'Triple Crown', 'Chester Thornless', and 'Prime-Jim'™ . Cuttings were 7 in. (18 cm) in length and averaged 1/3 in. (7.5 mm) in diameter, and were taken from the woody portion of the main stem. After collection in the field, cuttings were transported within an ice-filled cooler to the laboratory, where ends of the fresh cuttings were sealed by dipping 1/2 in. (1 cm) of each end in molten wax held at 100°C. After sealing they were briefly surfaced sterilized by submersion in a 1.5% sodium hypochlorite solution, wiped dry, and rinsed with water. Cuttings were then stored in a walk-in cooler overnight until inoculation the next day. The trial was repeated four times, with material collected on 10 and 24 Aug. 2002 and 18 July and 15 Aug. 2003.

Squares of media (~3 mm square or 13 in.) containing fungal mycelia were cut and placed in an incision 3 mm (1/8 in.) wide and 12 mm (1/2 in.) long made near the middle of each cutting. Incisions were deep enough to penetrate the epidermis and expose the underlying tissue without causing major vascular damage. After placing the inoculum, the wound was wrapped gently with a small piece of Parafilm® (American Can Company, Greenwich, Conn.) to hold the media in place and prevent desiccation of the incision. In each trial, two additional cuttings of each cultivar received uninoculated squares of PDA with streptomycin as controls.

The inoculated cuttings were placed in perforated plastic trays with clear plastic covers, suspended above water at a depth of approximately 1 in. (2 cm) to maintain humidity. The tray covers were misted with water once daily. Cuttings were placed diagonally on test tube racks in the center of the tray, and arranged according to a completely random design. The number of cuttings (replications) of each cultivar varied among trials. The 10 Aug. 2002 trial included eight of each genotype, the 18 July 2003 trial included 10 of each, and the 24 Aug. 2002 and 15 Aug. 2003 trials each had 12 of each cultivar.

Ten days following inoculation, the length and width of each resulting lesions was measured, and an area estimated by assuming an elliptical shape. The data were then transformed by taking the square root of this area, to compensate for skewness. Pair-wise student's t-test for mean separation and analysis of variance were performed using the JMP 5.0 statistical software package (SAS Institute, Cary, N.C).

Results and discussion

Fire Blight Resistance Study. Analysis of variance indicated that cultivar was the primary source of variance in the study, and that neither trial date nor replication had a significant effect. As such, data were summarized across trials. Significant differences were noted among many of the cultivars evaluated, indicating a wide range of resistances to the fire blight pathogen (Table 1). The cultivars Triple Crown, Arapaho, Prime-Jim™, and Kiowa all appeared highly resistant, with average disease severity ratings of less than 1. 'Navaho' was the most susceptible, with an average rating of 3.9. 'Navaho' is also a parent of two other susceptible cultivars, 'Apache' and 'Ouachita'. No symptoms were observed on any of the control plants.

Differences in genetic resistance to fire blight in blackberry were not unexpected. Studies have revealed both resistant and susceptible individuals in many host species of *E. amylovora*, including apple (Gardner et al., 1980), pear (Oitto et al., 1970) and red raspberry (Ries and Otterbacher, 1977). However, this study may be the first to document resistance to the pathogen in blackberry.

Botryosphaeria Cane Canker Resistance Study. Cultivar was shown by analysis of variance to be the only significant factor affecting lesion area, with no significant effects of trial or trial by genotype interaction. Significant differences in mean transformed lesion area were noted among the cultivars in the study (Table 2), on all trial dates. 'Chickasaw' was the most susceptible and was significantly greater than 'Ouachita', the second most susceptible. 'Arapaho' and 'Triple Crown' were once again among the most resistant, but 'Kiowa' was among the most susceptible cultivars. Most cuttings of 'Arapaho' and 'Triple Crown' showed no symptoms at all (data not shown).

It would seem likely, though not necessarily certain, that the differences observed in this detached cane assay would extend to intact plants. Smith (1988), working with rabbiteye blueberries (*V. ashei* Reade), found that a similar technique yielded the same ratings in both cuttings and potted plants. This detached-cane assay seems to confirm the resistance observed in both greenhouse and field plantings of 'Chester Thornless' noted by Maas (1986) and Maas and Uecker (1984).

Conclusions

Resistance to both *E. amylovora* and *B. dothidea* existed among the blackberry cultivars used in this study. Because both of these diseases have previously received relatively little study in blackberry, this information may prove useful for growers and researchers in the future. As the cultivation of blackberries expands to new regions and larger acreages, the potential exists for both these diseases to become more serious issues, and this study may help in the management of such problems.

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Table 1. Mean disease severity rating (0-5[z]) of blackberry genotypes 10 d after inoculation with *E. amylovora* .

Genotype	Mean rating
Navaho[y]	3.9 a[w]
Apache[y]	2.6 b
Ouachita	2.0 bcd
Shawnee	1.8 bcde
Chickasaw	1.5 cdef
Illini Hardy[y]	1.4 cdef
Chester Thornless[y]	1.1 def
Triple Crown[y]	0.9 ef
Arapaho[y]	0.8 ef
Prime-Jim™ [y]	0.8 ef
Kiowa	0.1 f

[z] Ratings scale: 0=no symptoms, 5=severe necrosis.

[y] Cultivar included in both trial 1 and trial 2 (n=11). All others included in trial 2 only (n=6).

[w] Means followed by the same letter do not differ significantly (*P* 0.05) according to the pair-wise student's t-test method.

Table 2. Mean of square-roots of *B. dothidea* lesion areas (cm^2) and mean areas for blackberr cuttings of different genotypes collected and inoculated on all dates.

Genotype	Mean area (transformed)	Mean area (cm2)
Chickasaw	2.49 a [z]	7.9
Ouachita	1.89 b	4.9
Kiowa	1.65 bc	4
Shawnee	1.65 bc	4
Prime-Jim™	1.63 bc	4.2
Navaho	1.59 bc	4.3
Apache	1.41 cd	3.4
Illini Hardy	1.40 bcd	2.8
Chester Thornless	1.07 d	2.4
Arapaho	0.31 e	0.6
Triple Crown	0.29 e	0.4

[z] Means followed by the same letter do not differ significantly (*P* 0.05) according to the pair-wise student's t-test method.