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Evaluation of post-harvest disease resistance in blackberry genotypes

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ABSTRACT

Forty-nine blackberry genotypes (19 cultivars and 30 breeding selections) were evaluated for post-harvest fruit-rot resistance in June and July 2003. Fully mature, undamaged berries were harvested on two dates for each genotype at the University of Arkansas Fruit Substation, Clarksville. After transporting in chilled coolers back to the Plant Pathology Department in Fayetteville, two replications of 10 berries of each genotype were placed in a high-humidity chamber for 3 d (21-23°C; 16-h daylength). This provided a total of four replications for each entry across the two harvest dates. Natural inoculum from the field provided the post-harvest pathogens, and no additional inoculations were conducted. Berries were evaluated after 3 d in the chambers for the presence of postharvest rot. If rot was present, then a rating scale of 1 to 3 (1-very little mycelial growth present; 3=berry totally covered by mycelia) was used to quantify rot. The fungal growth was examined visually and microscopically to identify the causal pathogen. There was a wide range of post-harvest fruit-rot responses among the genotypes. The cultivars with the least rot were 'Kiowa', 'Triple Crown', and A-1689, with 80%, 73%, and 60% of berries free of any rots, respectively. *Botrytis cinerea* was identified on all berries that had any presence of rot and was the most important pathogen that contributed to berry decay. *Colletotrichum* spp. was found less frequently on rotted berries. Results indicate that substantial fruit-rot resistance existed among genotypes and variation for resistance could likely be used in breeding. *Botrytis cinerea* is the primary pathogen to target in post-harvest fruit-rot breeding resistance at this study location.

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MEET THE STUDENT-AUTHOR

John-Paul Kidd

I am a native of Eureka Springs, Ark., but graduated from Fayetteville High School in 1996. I traveled throughout Australia and New Zealand after graduation and upon my return started school at the University of Arkansas. I quickly became interested in horticulture and the opportunities it could provide and decided to declare it as my major. After getting to know some of the horticulture professors it was suggested that I do a research project. I decided on blackberries and wanted to investigate the levels of post-harvest disease resistance among the different genotypes being developed by the University of Arkansas. This research project was especially appealing to me for two reasons: it was essentially an organic project free from pesticides, fungicides, and herbicides, and the fact that there was no literature associated with post-harvest disease resistance among blackberries meant that the project had the potential to provide useful information to the industry. I received the Katherine Bollenbacher Scholarship, the Mitchner Family Undergraduate Research Award, and a Dale Bumpers College of Agricultural, Food, and Life Sciences Undergraduate Research Grant to conduct this research project.

Dr. John R. Clark was my faculty advisor and encouraged me to do this research project to help me prepare and gain experience for graduate school. Also, Dr. Patrick Fenn of the Plant Pathology Department and Dr. Barbara Smith of the USDA-ARS small fruits research station in Poplarville, Miss., greatly helped me understand the processes of research. The results from this research were presented at the 64th Annual Meeting of the Southern Regional American Society for Horticultural Science in Tulsa, Okla., and at the 50th Annual Gamma Sigma Delta Honors Society competition; it won second place at both competitions.

INTRODUCTION

Blackberry (*Rubus* subgenus *Rubus*) production is important in Arkansas, other states in the U.S., and many countries around the world. The University of Arkansas is one of the leading establishments in the world for blackberry improvement. Thirteen cultivars have been released from the Arkansas program through 2004.

Although blackberries are resistant to most diseases, fruit rots do occur in some years. Recent observations made by Dr. Barbara Smith, USDA-ARS, indicated that fruit-rot occurrence varied in blackberry genotypes. The development of post-harvest disease- resistant varieties of blackberries is now becoming more of a focal point for researchers due largely to the expansion of shipping of blackberries. *Botrytis cinerea*, the fungus causing gray mold, is likely the primary disease causing post-harvest rot of ripe blackberries (John R. Clark, personal communication). It is the most important pathogen affecting blackberry production and marketing worldwide (Jarvis, 1962). Since previous research on the susceptibility of small fruits to *Botrytis* has been focused primarily on raspberries and strawberries, there are practically no publications related to the genotypic variance of blackberries and their related levels of resistance or susceptibility to post-harvest pathogens.

The fungicides that can be used to control *Botrytis* are recommended to be applied to berries three or four times a season. These materials are expensive and may be ineffective in wet seasons (Jarvis and Hargreaves, 1973). The mediocre disease control and a rise in public concern of the health hazards associated with fungicides has prompted the scientific community to search for alternative, perhaps more effective, means of disease control. Scientists are attempting to improve the effectiveness of disease control through genetic resistance in order to reduce fungicide dependency. If cultivars can be developed that have both good gray mold resistance and high-quality berries, the marketing of fresh fruit to

places distant from the centers of production could expand and production costs be reduced.

The main objectives of this research project were to evaluate multiple blackberry genotypes for resistance to post-harvest decays and to confirm which pathogen causes these losses. Then, if differences could be established, these may provide insight into the genetic variability of fruit-rot resistance and could also be useful in identifying resistance for use in breeding.

MATERIALS AND METHODS

Berries were harvested from 49 blackberry genotypes growing at the University of Arkansas Fruit Substation, Clarksville. The genotypes selected for evaluation included 13 Arkansas-developed cultivars, six cultivars developed elsewhere, and 30 breeding selections. The plants sampled were sprayed once with an application of liquid lime-sulfur at bud-break in March for anthracnose control. Berries were not washed after being picked. All inoculum provided was that which occurred naturally on the berries from the field. Samples were collected from late May to late July, 2003. Twenty fully ripe berries with no visible signs of disease or injury were selected at two harvest dates for each genotype. The berries were transported to the Plant Pathology Department at the University of Arkansas, Fayetteville, in coolers. The berries were then divided into two groups of 10 berries and placed into separate small trays lined with dry paper towels. These trays were then placed onto large black flats (six trays per flat). These flats were then placed into inverted, clear propagation domes that were lined with moist paper towels to keep the relative humidity near 100% without the fruit being in direct contact with moisture. The flat was then covered with another clear dome and sealed with tape to provide a tight, high-moisture chamber. The clear cover allowed observation of fruit-rot development without opening the container.

Fruit was incubated at 21°C-23°C in a room with a 16 h day (Smith et al., 1996). This environment provided optimal conditions for disease development on the fruits. Berries were rated for fruit rot 3 d after beginning of incubation. Number of berries with no rot present was recorded, and those with fruit rot were rated on a scale of 1-3—a value of 1=very little mycelial growth and 3=berry totally consumed by fungal growth. Evaluation of the fungi causing the fruit rot was determined by visual examination. The main fungi that were expected to be seen were *Botrytis cinerea* and *Colletotrichum* spp., (Barbara J. Smith, personal communication). However, the fruits were examined for other pathogens and their presences were recorded. A microscope viewed at 40x was used to identify and differentiate the pathogens. After all ratings were completed, the data were analyzed by analysis of variance using SAS (release 8.2) Proc GLM program. Mean separation was by LSD (P=0.05).

RESULTS AND DISCUSSION

There was a wide range of responses for the genotypes for all variables measured. None of the genotypes evaluated displayed a complete resistance to any of the post-harvest rots (Table 1). The genotypes with the highest resistance to fruit rots expressed as the percentage of fruit without evidence of rot included 'Kiowa' (80%), Navaho (72%), Triple Crown (60%), and A-1689 (60%). Of the 49 genotypes evaluated, 27 numbered selections rated only from 0% to 60% for fruit free of rot. Breeding selection A-2117 was the only genotype evaluated that had 0% fruit free of rot, showing the greatest susceptibility to postharvest fruit decay.

Results of the pathogen identification indicated that *Botrytis* contributed more than the *Colletotrichum* to total rot of the berries. *Botrytis* was observed on berries of all genotypes in the study (Table 1), while *Colletotrichum* was found on 40 of 49 genotypes (data not shown). However, the percentage of berries with *Colletotrichum* was much lower that *Botrytis*. Thus, *Botrytis* can be assumed to be the primary causal pathogen of the post-harvest rot. Other pathogens found were *Rhizopus* spp. and *Pestalotia*. Although their presences were recorded, their occurrences were too minute to contribute to fruit decay of any importance.

The fruiting season in which this research was conducted had an unusual amount of rain, and this could have affected some of the fruit responses. However, this climate should have contributed to even stronger pathogen pressure on the fruits.

These data are from a single season, thus repeated observations for additional years would be advantageous to verify the year-to-year stability of the fruit-rot resistance responses. This would allow the identification of superior genotypes for breeding and increasing the fruit-rot resistance genes.

The evaluation method used appeared to work very well in that the fruits had a very stable environment for pathogen development, and also the natural inoculum appeared to be uniform enough among genotypes and sample dates to provide a good pathogen pressure.

These data may prove helpful to growers who have high occurrences of fruit rots. Growers with high occurrences of these fruit rots can see that growing more resistant genotypes on their farms and avoiding growing others with high occurrences of fruit rots could lead to higher profits and lower production costs.

ACKNOWLEDGMENTS

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Table 1. Post-harvest fruit-rot severity occurrence from causal pathogens on 49 blackberry genotypes following incubation of fruits at 21-23°C for 3 d, 2003, Fayetteville, Ark. Breeding selections designated with "A" in the genotype identification are not available in commerce.

Genotype	% berries with no rots[z]	Average *Botrytis* rating[y]
Kiowa	80 a[x]	0.2 t
Navaho	72 ab	0.4 tu
Triple Crown	60 a-c	0.7 q-u
A-1689	60 a-c	0.5 s-u
A-1844	52 b-d	0.6 r-u
Apache	47 c-e	0.8 o-t
A-2005	45 c-f	0.6 r-u
Chester	45 c-f	0.7 p-u
A-830	45 c-0f	0.9 l-t
A-2035	42 c-g	0.8 o-t
A-1905	42 c-g	0.9 m-t
A-2255	37 c-h	1.0 k-t
Arapaho	37 c-h	0.8 o-t
A-2179	35 d-i	0.9 n-t
A-1790	32 d-j	1.0 j-s
A-1942	30 d-k	1.3 e-q
Cherokee	27 e-l	1.3 e-q
Illini Hardy	27 e-l	1.1 h-r
Darrow	26 e-m	1.4 b-n
A-1981	25 e-m	1.3 e-q
A-2047	25 e-m	1.2 f-q
A-2078	23 e-n	1.1 i-s
A-2091	23 e-n	1.4 c-o
A-2241	22 f-n	1.2 f-q
A-2143	20 g-n	1.2 g-r
A-2212	20 g-n	1.3 d-p
Shawnee	20 g-n	1.6 b-k
A-2151	15 h-n	1.6 a-k
A-2141	15 h-n	1.8 a-e
Humble	15 h-n	1.8 a-f
Choctaw	12 i-n	1.9 a-d
A-2095	12 i-n	1.5 b-m
A-2156	12 i-n	1.3 e-q
A-2064	12 i-n	1.5 b-l
A-2009	12 i-n	1.5 b-m
A-2221	10 j-n	1.3 d-p
A-2200	10 j-n	1.6 a-j
APF-15	10 j-n	1.3 c-o
Chickasaw	10 j-n	1.5 b-k
Prime-Jan	10 j-n	1.6 a-k
Prime-Jim	7 k-n	2.2 a
A-2046	7 k-n	1.7 a-i
A-2177	6 k-n	1.7 a-g
Cheyenne	5 l-n	1.6 b-k
Rosborough	5 l-n	2.0 ab
Comanche	3 l-n	2.0 ab
A-2150	2 mn	1.6 a-h
A-1695	2 mn	1.8 a-f
A-2117	0 n	1.9 a-c

[z] The average number of berries out of a 10-berry sample which showed no post-harvest rot after 3-d incubation, based on four samples rated.

[y] The average disease rating of a 10-berry sample where *Botrytis* was the primary pathogen on a disease severity scale of 1= very little mycelial growth; 3 = berry totally consumed by fungal growth; based on four samples rated.

[x] Means not followed by the same letter differ significantly ($P \leq 0.05$) as determined by LSD.