Susceptibility of Rabbiteye Blueberry Cultivars to Postharvest Diseases

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ABSTRACT

Smith, B. J., Magee, J. B., and Gupton, C. L. 1996. Susceptibility of rabbiteye blueberry cultivars to postharvest diseases. Plant Dis. 80:215-218.

Thirteen rabbiteye cultivars were surveyed for postharvest berry rots during the 1992, 1993, and 1994 harvest seasons. Disease incidence and severity were low, with <30% of berries displaying decay symptoms after 5 days incubation at 25°C with 100% relative humidity (RH). Although Botrytis fruit rot and ripe rot were equally severe in 1992, ripe rot was the most common postharvest disease in 1993 and 1994. Menditoo, Homebell, Beckyblue, and Premier blueberries had more than 40% rotted fruit, while Briteblue, Southland, and Tifblue had less than 16%. The pathogen associated with ripe rot was identified as *Colletotrichum acutatum* rather than the more commonly reported pathogen, *C. gloeosporioides*. When ripe berries from all 13 cultivars were inoculated with a conidial suspension of *C. acutatum* and incubated for 5 days at 25°C and 100% RH, all were infected with ripe rot. Tifblue, Delite, Premier, and Menditoo scored the highest for ripe rot, while Southland, Centurion, Woodard, Bluebell, and Homebell scored the lowest. Generally, inoculated berries from late-season harvests scored higher for ripe rot than did berries from earlier harvests. Two of the cultivars most susceptible to ripe rot, Tifblue and Premier, account for most of the rabbiteye blueberry production in the southeastern United States.

Blueberries (*Vaccinium* spp.) are a major North American fruit crop. Their production is steadily increasing, particularly in the southeastern United States, where the area planted to rabbiteye blueberries (*V. ashei* Reade) more than doubled between 1982 and 1992 (11). Rabbiteye blueberries traditionally have been hand-harvested and marketed locally, but today more berries are being machine-harvested and shipped to distant markets to be sold fresh. Berry diseases and postharvest fruit rots have not been a major problem of rabbiteye blueberries (1), but as the blueberry industry expands, fruit diseases will increase in importance.

The most important postharvest fruit rots of blueberry (rabbiteye and highbush, *V. corymbosum* L.) include Botrytis rot or gray mold (*Botrytis cinerea* Pers.:Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel)), Alternaria rot (*Alternaria tenuissima* (Kunze:Fr.) Wiltshire), anthracnose fruit rot or ripe rot (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk) and *Colletotrichum acutatum* J.H. Simmonds), and Phomopsis rot (*Phomopsis vaccinii* Shear in Shear, N. Stevens, & H. Bain (teleomorph *Diaporthe vaccinii* Shear)) (3,7,8,10,14). Little information is available on the relative importance of these diseases on rabbiteye blueberry or on the susceptibility of the major rabbiteye cultivars to these diseases. The purpose of this study was to determine which diseases were occurring on harvested fruit of several rabbiteye cultivars, the relative susceptibility of these cultivars to ripe rot, and the identity of the *Colletotrichum* spp. causing ripe rot in the southeastern United States.

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Accepted for publication 14 November 1995.

Publication no. D-1995-1227-05R

MATERIALS AND METHODS

Noninoculated berries. Berries used in this study were harvested from 13 rabbiteye cultivars established in 1979 at the USDA-ARS Small Fruit Research Station in Poplarville, MS, on a Ruston fine sandy loam soil. The design of the planting was a randomized complete block with four blocks and two plants per block. The plants were maintained as recommended by Braswell et al. (2) and were irrigated as needed by overhead sprinkler irrigation. No fungicides were applied to the plants in this trial during the 3-year study. Following the 1992 harvest, the plants were pruned back to 0.5 m.

Early-ripening berries were collected and incubated in moist chambers (140-mm petri dishes lined with moist filter papers), and a number of pathogens were isolated from decaying berries onto acidified (pH 3.5) potato-dextrose agar (APDA). From this group of pathogens, an isolate (TB-Pop) of the ripe rot fungus, *C. acutatum*, was cultured on potato-dextrose agar (PDA) under continuous fluorescent light at 25 ± 1°C. Seven- to ten-day-old cultures of this isolate were used to prepare suspensions of 5×10^5 conidia per ml to inoculate harvested fruit.

Inoculation experiments. Ripe berries were hand-picked in 1992, 1993, and 1994 at 1-week intervals beginning at approximately midseason for each cultivar and continuing for 4 weeks. Twenty ripe, unblemished berries were selected from each of four bushes of each cultivar at each harvest, randomly separated into two groups of 10 berries each, and placed calyx side down in 90-mm petri dishes. Berries in one group were inoculated by placing 0.1 ml (50,000 conidia) of a conidial suspension of *C. acutatum* into the stem scar of each berry. Berries in the second group were not inoculated. Both groups of berries were incubated at 25 ± 1°C for 5 or 6 days in a moist chamber consisting of a large pan (40 × 50 cm) lined with moist paper towels and covered with plastic wrap.

Following incubation, each berry was observed under low power magnification and rated for the presence of fungal pathogens. For each pathogen, disease incidence for each berry was scored as 0 = no symptoms, 1 = symptoms visible only with magnification, 2 = symptoms visible to trained observer without magnification, and 3 = severe symptoms. Soft, leaky, or otherwise defective berries whose symptoms were not associated with a particular disease were scored as "unknown." Isolations were made on APDA plates from several berries at each rating to confirm the identity of each pathogen.

Identification of ripe rot pathogen. Because ripe rot was the most prevalent postharvest disease, isolations were made from at least 25 noninoculated berries with ripe rot symptoms during each of the 1992 to 1995 harvest seasons. Twenty-two isolates of *Colletotrichum* spp. obtained from ripe rabbiteye blueberry fruit harvested at Poplarville in 1995 and designated by the prefix BBPop were compared with four other isolates from blueberry fruit (DollBB25, DollBB26, Blueberry 20, and TB-Pop) and two isolates from blueberry leaves (FlaBB and FlaBB408) obtained from Florida. Conidial shape and size, presence of setae, production of perithecia, and colony characteristics of each blueberry isolate were compared with *C. gloeosporioides* isolate CG-162 and *C. acutatum* isolate Goff (13). Colony color was determined on 5-day-old cultures grown at 25 ± 1°C on PDA. Forty conidia from 10- to 20-day-old PDA cultures were measured, and their shapes were rated as fusiform (both ends abruptly tapered), boat-shaped (one end tapered, one end rounded), or cylindrical (both ends rounded). Colony growth was rated after 5 days growth on PDA at 30°C in the dark.

The SAS statistical package (SAS Institute, Cary, NC) was used for analysis of variance. Treatment means were separated by the least significant difference.

RESULTS

Noninoculated berries. Overall, both incidence and severity of postharvest decay on berries of the 13 rabbiteye cultivars tested were low (Table 1). After 5 days incubation at 25°C and 100% relative humidity (RH), only 33 and 36% of the berries from the 1992 and 1994 seasons, respectively, exhibited any decay symptoms; significantly fewer (17%) berries from the 1993 harvest were decayed. The total fruit rot score was lowest in 1993 (0.33) and highest in 1994 (0.75). Ripe rot caused the largest proportion of decay in 1993 and 1994. Although ripe rot and Botrytis fruit rot were equally severe in 1992, Botrytis fruit rot was insignificant in 1993 and 1994. Other pathogens identified as causing postharvest rots included *Alternaria*, *Phomopsis*, *Rhizopus*, and *Mucor* spp.

The percentage of berries with decay symptoms from all causes was lowest (23 to 29%) at the three early harvests and highest (41%) at the last harvest. This increase was due primarily to the increase in ripe rot incidence. Ripe rot disease scores were significantly lower (0.17 to 0.38) at the early harvests than at the last harvest (0.59). Botrytis rot disease scores decreased with each harvest, from 0.12 at the first harvest to 0.05 at the last harvest. In 1992, Botrytis fruit rot was more severe at the first two harvests than at the last two harvests ($P = 0.05$).

Menditoo, Homebell, Beckyblue, and Premier had the greatest incidence (>40%) of rotted fruit and the highest total fruit rot score (>0.9) of the 13 cultivars (Table 2). Briteblue, Southland, and Tifblue had the lowest incidence of rotted fruit (<16%) and, along with Bluebell, the lowest total fruit rot score (<0.4). Homebell, Menditoo, and Premier had the most severe ripe rot, while Briteblue, Climax, Southland, and Bluebell had the least severe ripe rot. Beckyblue, Climax, and Premier had the most Botrytis fruit rot. Beckyblue also had the highest amount of other rots (Table 2).

Inoculated berries. Following inoculation with *C. acutatum*, all cultivars developed typical ripe rot symptoms. *C. acutatum* could always be reisolated from inoculated berries. Tifblue, Delite, Premier, and Menditoo had the highest ripe rot scores (Table 3), while Southland, Centurion, Woodard, Bluebell, and Homebell had the lowest ripe rot scores. Ripe rot scores were significantly higher on inoculated berries collected at the fourth harvest (2.16) than on those collected earlier in the season (1.63 to 1.90). The severity of Botrytis fruit rot and other rots on berries inoculated with *C. acutatum* (Table 3) was similar to the severity on noninoculated berries (Table 2).

Identification of ripe rot pathogen. Twenty-four *Colletotrichum* isolates from fruit were identified as *C. acutatum*, and two were identified as *C. gloeosporioides* (Table 4). Both *Colletotrichum* isolates from leaves obtained from Florida were identified as *C. gloeosporioides.* All isolates produced hyaline, straight conidia. The shape of the conidia of the *C. gloeosporioides* isolates was variable, but less than half were fusiform. The *C. acuta-*

Table 1. Average disease severity ratings and percent berries with postharvest decay of 13 rabbiteye blueberry cultivars by year

Year	Ripe rot[x]	Botrytis rot	Other rots[y]	Total disease	Total rots (%)
1992	0.21 b[z]	0.20 a	0.23 a	0.65 b	33 b
1993	0.17 b	0.01 b	0.15 b	0.33 c	17 a
1994	0.58 a	0.03 b	0.14 b	0.75 a	36 b

[x] Diseases scored on scale of 0 = no symptoms to 3 = berry completely rotted. Scores are averages of 10 berries per replication, four replications per harvest, and four harvests per year. Berries were not inoculated after harvest, but were incubated at 25°C and 100% relative humidity for 5 to 6 days before scoring.
[y] Other rots include *Phomopsis*, *Mucor*, *Rhizopus*, and *Alternaria* spp., and unknown causes.
[z] Mean separation within columns by LSD, $P = 0.05$.

Table 2. Average disease rating and percent rotted berries of 13 rabbiteye blueberry cultivars for 1992, 1993, and 1994 seasons at Poplarville, MS

Cultivar	Ripe rot[x]	Botrytis rot	Other rots[y]	Total disease	Total rots (%)
Menditoo	0.65 ab[z]	0.10 cd	0.25 bc	1.00 ab	46.98 a
Homebell	0.74 a	0.06 de	0.24 bc	1.05 a	46.77 a
Beckyblue	0.38 cd	0.18 a	0.39 a	0.95 ab	45.62 a
Premier	0.59 b	0.13 a-c	0.20 b-d	0.93 ab	40.87 ab
Delite	0.45 c	0.12 bc	0.29 b	0.86 b	38.30 b
Woodard	0.25 ef	0.04 e	0.23 bc	0.52 c	29.04 c
Powderblue	0.30 de	0.06 de	0.12 de	0.48 cd	25.00 cd
Climax	0.10 gh	0.15 ab	0.17 c-e	0.43 c-e	24.17 cd
Centurion	0.23 e-g	0.05 e	0.12 de	0.39 c-f	21.44 de
Bluebell	0.16 f-h	0.05 e	0.12 de	0.33 d-g	19.02 d-f
Tifblue	0.20 e-g	0.04 e	0.07 e	0.31 e-g	15.84 e-g
Southland	0.13 f-h	0.04 e	0.07 e	0.24 fg	13.40 fg
Briteblue	0.07 h	0.03 e	0.09 e	0.19 g	11.43 g

[x] Diseases scored on scale of 0 = no symptoms to 3 = berry completely rotted. Scores average of 10 berries per replication, four replications per harvest, and four harvests per year. Berries were not inoculated after harvest, but were incubated at 25°C and 100% relative humidity for 5 to 6 days before scoring.
[y] Other rots include *Phomopsis*, *Mucor*, *Rhizopus*, and *Alternaria* spp., and unknown causes.
[z] Mean separation within columns by LSD, $P = 0.05$.

Table 3. Average disease severity rating and percent rotted berries following inoculation with *Colletotrichum acutatum* of 13 rabbiteye blueberry cultivars for 1992, 1993, and 1994 seasons at Poplarville, MS

Cultivar	Ripe rot[x]	Botrytis rot	Other rots[y]	Total disease	Total rots (%)
Tifblue	2.14 a[z]	0.04 cd	0.36 a	2.55 a	93.0 ab
Premier	2.04 ab	0.12 ab	0.32 a	2.49 ab	95.3 a
Delite	2.06 ab	0.07 b-d	0.35 a	2.49 ab	92.7 ab
Menditoo	2.02 a-c	0.16 a	0.17 c	2.36 a-c	92.3 ab
Climax	1.90 b-d	0.08 b-d	0.30 ab	2.29 b-d	89.1 a-d
Beckyblue	1.89 b-d	0.09 a-d	0.41 a	2.41 ab	89.6 a-d
Briteblue	1.89 b-d	0.09 b-d	0.17 c	2.16 c-e	90.8 a-c
Powderblue	1.84 c-e	0.08 b-d	0.15 c	2.08 d-f	89.2 a-d
Homebell	1.75 d-f	0.11 a-c	0.20 bc	2.07 d-f	85.0 c-e
Bluebell	1.73 d-f	0.10 a-d	0.18 c	2.01 ef	89.2 b-e
Woodard	1.73 d-f	0.04 cd	0.18 c	1.96 ef	87.3 b-e
Centurion	1.66 ef	0.06 b-d	0.18 c	1.90 f	82.5 e
Southland	1.61 f	0.03 d	0.21 bc	1.86 f	84.5 de

[x] Berries inoculated with 5×10^5 conidia per milliliter suspension of *C. acutatum* and incubated for 5 to 6 days at 25°C and 100% relative humidity. Diseases scored on scale of 0 = no symptoms to 3 = berry completely rotted. Scores average of 10 berries per replication, four replications per harvest, and four harvests per year.
[y] Other rots include *Phomopsis*, *Mucor*, *Rhizopus*, and *Alternaria* spp., and unknown causes.
[z] Mean separation within columns by LSD, $P = 0.05$.

tum isolates produced mostly fusiform conidia that on average were significantly smaller than the *C. gloeosporioides* conidia (Table 5). In contrast, *C. gloeosporioides* isolates produced conidia of which 77% were boat-shaped or cylindrical. All the *C. acutatum* isolates produced abundant masses of conidia and orange, pink, or lavender colonies that were slightly lighter in color when viewed from the reverse. *C. gloeosporioides* isolates produced masses of orange conidia that were usually covered by the olive-gray, cottony, aerial mycelium. The reverses of these colonies were creamy gray-green. The isolates of *C. gloeosporioides* produced a few setae and perithecia on 10- to 20-day-old PDA cultures; however, none of the *C. acutatum* isolates produced obvious setae or perithecia on PDA cultures. At 30°C, the *C. acutatum* isolates grew at a significantly slower rate than did the *C. gloeosporioides* isolates (Tables 4 and 5). Based on these characteristics, the causal fungus was identified as *C. acutatum* J. H. Simmonds (12,13). *C. gloeosporioides* (teleomorph *G. cingulata*) has previously been reported to cause anthracnose fruit rot or ripe rot (5,6,8,10) of blueberries; however, this species was isolated from only two berries in this study and is not usually isolated from blueberries with ripe rot symptoms in the south Mississippi area.

DISCUSSION

To our knowledge, this is the first study to compare the susceptibility of several rabbiteye blueberry cultivars to various postharvest diseases. Generally, rabbiteye blueberries are considered to be more resistant to diseases than highbush blueberries (1). However, we found few reports that directly compared several cultivars of each species (4,5,9). Highbush cultivars were not included in this study because they are not adapted to the climate of south Mississippi.

Based on this study and observations of berries from commercial fields, fruit rot diseases are not causing significant preharvest or postharvest losses of rabbiteye blueberries in Mississippi at present, even though most plantings are not sprayed with fungicides. In North Carolina (4,9) Alternaria fruit rot is an important postharvest fruit rot of highbush and rabbiteye blueberries; however, the amount of Alternaria fruit rot in our studies was extremely low. Ripe rot and Botrytis fruit rot were the most important fruit diseases found in our studies and have been reported to be the third and fourth most important diseases of blueberry, respectively (11). In North America, ripe rot losses of blueberries before harvest are estimated at 3 to 5%; but during shipment and storage, losses may approach 100% in some instances when a single diseased berry infects an entire container of fruit (8). Mummy berry causes more crop loss at harvest in most areas than any other disease (10); however, this disease, which was confirmed for the first time in commercial rabbiteye plantings in Mississippi in 1995, was not present in our studies.

The low incidence and severity of fruit rots among the rabbiteye blueberry cultivars are probably due to their resistance to fruit diseases, particularly when compared to highbush cultivars. Daykin and Milholland (5) found the rabbiteye cultivar Powderblue had fewer berries with ripe rot (1%) at harvest than seven highbush cultivars (3 to 26%). In our study, Powderblue was midway in susceptibility to ripe rot among the 13 rabbiteye cultivars. The

Table 4. Conidial and colony characteristics of 28 isolates of *Colletotrichum* spp. from blueberry compared with two *Colletotrichum* spp. isolated from strawberry

Species Isolate	Conidial size[v] (μm) Length	Width	Percent[w] fusiform	Growth[x] (mm/day)	Colony color Top	Reverse
C. gloeosporioides						
BBPop1	16.00 a-c[y]	6.02 a-c	38 c	12.7 ab	Light olive gray	Greenish cream
BBPop21	14.89 c-h	5.50 b-e	3 d	11.6 b	Olive green	Olive cream
FlaBB	15.11 b-f	6.13 b	28 d	13.5 a	Light gray	Creamy yellow
FlaBB408	15.22 b-e	5.88 a-c	25 cd	12.9 ab	White	White
CG162[z]	16.17 ab	5.85 a-d	40 c	12.4 a-c	Gray	Olive cream
C. acutatum						
BBPop2	14.45 d-i	5.63 b-e	100 a	7.1 c-g	Creamy orange	Creamy orange
BBPop3	14.33 d-i	5.71 b-d	100 a	6.7 d-g	Peachy orange	Medium orange
BBPop5	14.47 d-i	5.61 b-e	100 a	6.4 d-g	Grayish orange	Cream
BBPop6	16.44 a	4.95 e-g	98 a	5.9 e-g	Grayish peach	Light orange
BBPop7	14.96 d-g	5.72 b-d	100 a	6.6 d-g	Medium orange	Creamy orange
BBPop8	14.21 e-i	5.56 b-e	100 a	6.4 d-g	Grayish orange	Creamy orange
BBPop9	14.57 d-i	5.66 b-e	100 a	5.9 fg	Pinkish lavender	Creamy orange
BBPop10	13.60 i-k	5.75 b-d	100 a	7.8 cd	Grayish lavender	Rosy beige
BBPop11	14.35 d-i	5.91 a-c	100 a	7.7 c-e	Grayish pink	Creamy orange
BBPop12	13.44 i-k	5.78 b-d	98 a	5.4 g	Grayish orange	Creamy salmon
BBPop13	13.74 h-k	5.60 b-e	90 ab	7.4 c-f	Orange	Creamy orange
BBPop14	13.46 i-k	5.13 d-g	93 a	6.9 c-g	Brownish orange	Yellow orange
BBPop15	13.46 i-k	6.53 a	88 ab	7.1 c-g	Pinkish lavender	Lavender orange
BBPop17	13.96 f-j	5.58 b-e	95 a	7.5 c-f	Peachy white	Creamy orange
BBPop18	15.03 b-f	5.78 b-d	100 a	8.5 c	Grayish pink	Creamy orange
BBPop19	15.03 b-f	5.67 b-e	98 a	6.5 d-g	Mauve	Dark rose
BBPop20	14.02 f-j	5.38 c-e	83 ab	6.1 d-g	Orange	Creamy orange
BBPop22	15.32 a-e	5.68 b-e	100 a	6.3 d-g	Gray cream	Creamy orange
BBPop23	14.92 d-h	5.71 b-d	95 a	6.0 e-g	Creamy pink	Creamy orange
BBPop24	14.61 d-h	5.77 b-d	100 a	6.5 d-g	Fuchsia pink	Dark orange
Blueberry20	12.75 k	4.63 fg	85 ab	6.1 e-g	Light orange	Creamy orange
DollBB25	12.93 jk	5.35 c-f	100 a	7.4 c-f	Dark orange	Creamy orange
DollBB26	14.47 d-i	5.88 a-c	95 a	7.4 c-f	Pinkish lavender	Creamy fuchsia
TB-POP	13.84 g-k	5.42 b-e	68 ab	5.9 e-g	Pinkish orange	Brownish orange
Goff[z]	15.39 a-d	4.47 g	93 ab	5.8 fg	Creamy orange	Creamy orange
LSD	1.19	0.74	26	1.7		

[v] Average measurement of 40 conidia taken from 10- to 20-day-old cultures grown on potato-dextrose agar at 25°C.
[w] Percentage of 40 conidia rated as fusiform (abruptly tapered on both ends).
[x] Average radial growth of four to eight replicates on potato-dextrose agar at 30°C. Colonies were measured after 5 days.
[y] Mean separation within column according to LSD ($P = 0.05$).
[z] Strawberry isolate (13) included for comparison.

Table 5. Average conidial characteristics and colony growth rate of four *Colletotrichum gloeosporioides* and 24 *C. acutatum* isolates from blueberry

Species	Conidial size[x] (μm)		Percent conidia with shape[y]			Growth[z] (mm/day)
	Length	Width	F	B	C	
C. gloeosporioides	15.48	5.87	23	46	31	12.6
C. acutatum	14.31	5.55	95	2	3	6.8
LSD	0.52	0.29	7	5	4	0.6

[x] Average measurement of 40 conidia taken from 10- to 20-day-old cultures grown on potato-dextrose agar at 25°C.
[y] Percentage of 40 conidia rated fusiform (tapered on both ends) = F, boat-shaped (tapered on one end, rounded on one end) = B, cylindrical (rounded on both ends) = C.
[z] Average radial growth of four to eight replicates on potato-dextrose agar at 30°C. Colonies were measured after 5 days.

difference in disease levels in this study from year to year probably was due to weather conditions, and in 1993 inoculum levels may have been reduced following severe pruning of the plants after the 1992 harvest. In addition, a late freeze in the spring of 1993 killed many flowers and reduced the early crop.

C. acutatum was determined to be the causal agent of ripe rot of the blueberries in this study. Over the past 10 years, numerous *Colletotrichum* spp. isolates from various hosts and locations have been collected at Poplarville. All isolates from blueberry fruit were identified as *C. acutatum* except for two obtained in 1995, which were identified as *C. gloeosporioides*. One of these isolates (BBPop21) was taken from the same fruit from which a *C. acutatum* isolate was also obtained. The two isolates on blueberry leaves from Florida were identified as *C. gloeosporioides*. We have routinely isolated *C. gloeosporioides* from blueberry leaves with typical anthracnose lesions; however, none of these isolates were maintained and included in this study.

The ripe rot disease scores of both noninoculated and inoculated berries were significantly greater at the last harvest than those in berries from earlier harvests. This increase in incidence and severity of ripe rot in later harvests is probably due to a buildup of inoculum on overripe berries remaining on the bushes, environmental conditions more favorable to ripe rot infection, and the increased susceptibility of fruit as it ripens.

The susceptibility of Tifblue and Premier to ripe rot is of concern, since 40% of the rabbiteye acreage is currently planted with Tifblue (11) and the area planted with Premier has been increasing steadily over the past 10 years. Together, these two cultivars account for most of the blueberry production in Mississippi and Louisiana. Premier has been promoted because it is early ripening; however, in addition to its susceptibility to ripe rot and other postharvest rots, it is softer at full ripeness than Tifblue (J. B. Magee, *unpublished data*), which may limit its potential for shipment to fresh markets. Tifblue ripens later than most rabbiteye cultivars, which makes it more suitable for processing.

ACKNOWLEDGMENTS

We thank Wanda S. Elliott for technical assistance and Alecia Whidden, University of Florida, Gulf Coast Research and Education Center, Dover, for providing cultures of FlaBB and FlaBB408.

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