Incidence and Pathogenicity of *Phyllosticta vaccinii* and *Botryosphaeria vaccinii* on Cranberry

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ABSTRACT

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Botryosphaeria vaccinii and *Phyllosticta vaccinii* were compared on the basis of incidence and pathogenicity to cranberry leaves and fruit. *P. vaccinii* was associated with preharvest fruit decay and was pathogenic to leaves, shoots, and fruit in growth chamber tests. Maximum disease severity on leaves and fruit occurred when plants were incubated at 28 C following a 72-hr dew period at 24 C. *B. vaccinii* established latent infections on naturally infected cranberry leaves and fruit and caused postharvest decay in fruit stored at 24 C. Inoculated fruit developed fruit speckle symptoms or decayed in growth chamber tests. *B. vaccinii* was found in samples from all the production areas surveyed, whereas *P. vaccinii* was recovered only from New Jersey and Massachusetts.

Additional key words: *Guignardia vaccinii*

Botryosphaeria vaccinii (Shear) Barr (*Guignardia vaccinii* Shear) has been reported to cause several diseases on cranberry (*Vaccinium macrocarpon* Ait.), including a blight of flowers and young fruit, preharvest fruit decay, postharvest storage rot, leaf spot (6,7), and a superficial spotting of the fruit epidermis known as speckle (2). Latent infections on cranberry leaves and fruit have also been attributed to this organism (3,4,6).

Recently, a new species, *Phyllosticta elongata* Weidemann, was described (12) as the anamorph of *B. vaccinii* on cranberry in place of *P. vaccinii* Earle, which was retained as a distinct species. Cultural and morphological characteristics of both species were described (12).

The taxonomic revision created a need to reassess the etiology of the various diseases previously attributed exclusively to *B. vaccinii*. This study was conducted to compare these two species on the basis of their distribution and incidence in naturally infected cranberry leaves and fruit and to determine their pathogenicity to cranberry leaves and fruit in growth chamber tests. A preliminary report of a portion of this work has been published (11).

MATERIALS AND METHODS

Cranberry leaf and fruit samples were obtained from the Blueberry and Cranberry Research Center, Rutgers University, Chatsworth, NJ 08019; the Cranberry Experiment Station, East Wareham, MA 02538; Gottschalk Cranberry Co., Inc., Wisconsin Rapids, WI 54494; and the Coastal Washington Research and Extension Unit, Long Beach, WA 98631. Samples were obtained of the more common varieties of the respective regions. Wisconsin samples were taken from the cultivar Searles and Washington samples from the cultivar McFarlin. New Jersey samples were commonly taken from Early Black or Franklin and Massachusetts samples from Early Black.

Cranberry leaves and decayed fruit were cultured when received. Sound fruit were separated into three equal groups and stored at room temperature (22–24 C), 10 C, and 4 C in aluminum baking pans (20 × 25 cm) loosely covered with paper bags. The samples were examined at 2-wk intervals for up to 25 wk, and the decayed fruit were removed for subsequent culturing.

Cranberry leaves were surface-disinfested for 5 min in 1% sodium hypochlorite plus one drop of Triton X-100/100 ml of solution, rinsed in sterile distilled water, and plated on potato dextrose agar (PDA). Decayed fruit were held in 70% ethanol for 15 sec, then quickly flamed to remove excess alcohol. A portion of the epidermis was resected near the margin of the lesion, and a small piece of parenchyma was removed and transferred to PDA. All cultures were incubated for 10–14 days at room temperature (22–24 C). Monoconidial stock cultures were maintained on PDA at 4 C.

Plants were propagated from cuttings of cranberry uprights (cv. Searles) collected while dormant from a commercial marsh in Wisconsin Rapids. Four cuttings were established in each 9-cm plastic pot containing peat, quartz sand, and unsterilized cranberry bog soil (1:1:1, v/v). The resulting plants were maintained in growth chambers (24 C, 16-hr photoperiod) and were pruned to 15–20 cm in height 4–6 wk prior to inoculation. Fruit set was obtained by hand-pollinating flowers. Full-sized, partially ripened fruit developed within 4–5 mo.

Inoculum was prepared from cultures grown on 9-cm cellophane squares on PDA plates. The cellophane was prepared as described by Rusmin and Leonard (5). After 12–14 days of incubation at room temperature (22–24 C), the cellophane and adhering colonies were removed and triturated in 50 ml of sterile distilled water, filtered through cheesecloth, centrifuged at 12,000 *g* for 20 min, and resuspended. Conidial suspensions were standardized at 1–2 × 10^5 spores per milliliter with a hemacytometer.

For leaf spot studies, plants were spray-inoculated with spore suspensions (isolates F4–F7 of *B. vaccinii* and isolates S2–S6 of *P. vaccinii*) with an atomizer and placed immediately into darkened dew chambers at 20 or 28 C. After 72 hr, the plants were transferred to a growth chamber (24 C, 16-hr photoperiod) for 6 wk. Each treatment was replicated three times.

To determine the optimum dew period temperature for infection of leaves, plants were inoculated with isolates S3 and S6 of *P. vaccinii* as previously described and immediately placed into darkened dew chambers at 16, 20, 24, 28, or 32 C. After 72 hr, half the plants were placed in a 20-C growth chamber (12-hr photoperiod) and the other half in a 28-C growth chamber (12-hr photoperiod). Leaf spot counts were made at weekly intervals for 6 wk.

For fruit inoculations, the inoculum of each species was composed of the combined spore suspensions from three recent isolates (isolates F9–F11 of *B. vaccinii* and isolates S7–S9 of *P. vaccinii*) to ensure optimal pathogenicity. Inoculated plants with fruit were placed in a darkened dew chamber at 24 C for 72 hr, then placed in 28- or 20-C growth chambers as previously described. As soon as symptoms developed, decayed fruit were removed, surface-disinfested, and cultured on PDA.

RESULTS

Incidence in cranberry fruit and leaves. *P. vaccinii* was often the most common

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pathogen isolated from preharvest decayed fruit samples from New Jersey and Massachusetts (Table 1). No differences in disease incidence or severity were noted among the cultivars sampled. *B. vaccinii* was never recovered from fruit that decayed before harvest. Conversely, *B. vaccinii* was commonly recovered from fruit that decayed in storage at room temperature (Table 2), whereas *P. vaccinii* was never recovered from postharvest decayed fruit. The number of *B. vaccinii* cultures recovered was considerably less from fruit incubated at 10 and 4 C than from fruit incubated at 24 C. *B. vaccinii* was isolated from fruit treated with protectant fungicides to prevent preharvest decay, as well as from untreated fruit.

B. vaccinii was commonly isolated from asymptomatic cranberry leaves collected in all the major cranberry-growing regions (Table 3), whereas *P. vaccinii* was recovered only from leaves collected in New Jersey and Massachusetts. Leaves from New Jersey with leaf spot symptoms yielded primarily *P. vaccinii*, whereas *B. vaccinii* was commonly isolated from symptomless leaves from the same sample. Occasionally, both fungi could be isolated from the same leaf segment. No leaf spot symptoms were noted on leaf samples collected in the other regions.

Pathogenicity to leaves. All isolates of *P. vaccinii* tested proved to be pathogenic to cranberry leaves at both 20 and 28 C in dew chamber tests except isolate S4, which was pathogenic only at 28 C. Plants inoculated with *B. vaccinii* isolates did not develop symptoms. Infection percentages by *P. vaccinii* were generally low, typically ranging from 1 to 5% of the inoculated leaves.

Leaf spot lesions with distinct brown or black margins developed on upper leaf surfaces 10–14 days after inoculation. Separate, black pycnidia appeared on the upper surface of the lesions 14–16 days after inoculation. In most cases, the lesions enlarged until the entire leaf was killed, although at times lesion development was limited by the midvein.

Occasionally, symptoms of shoot blight, a condition not previously reported, were noted on inoculated plants. Small necrotic stem lesions developed within 10–14 days of inoculation, and pycnidia appeared on diseased areas within 14–16 days. The stems were eventually girdled, killing the stem tissue distal to the lesion. Lesions were found only on juvenile stem tissue near the shoot apex and never developed on mature woody tissues. Similar leaf spot symptoms and several uprights with shoot blight symptoms identical to those formed on the inoculated material were observed on the sample of plant materials from New Jersey.

Disease severity on leaves was greatest in treatments receiving a 72-hr dew period incubation at 24 C and postinfection incubation at 28 C (12-hr photoperiod) (Fig. 1). Plants maintained at 20 C in growth chambers usually had significantly fewer lesions. Leaf spot symptoms were first noted at 2 wk on plants maintained at 28 C, whereas symptom development at 20 C was first recorded at 3 wk. Mature leaves were much less likely to develop lesions at either 20 or 28 C and those that

Table 1. Incidence of *Phyllosticta vaccinii* and *Botryosphaeria vaccinii* in field-rotted cranberry fruit

Sample source[a]	Year	No. of fruit sampled	Percentage of sample decayed by: *P. vaccinii*	*B. vaccinii*	Other fungi[b]
New Jersey	1975	40	74	0	27
New Jersey	1976	200	77	0	24
New Jersey	1977	81	53	0	49
New Jersey	1978	103	33	0	67
Massachusetts	1975	63	55	0	45
Massachusetts	1976	140	30	0	71

[a] New Jersey samples were obtained from the cultivars Early Black and Franklin, except in 1978 when a nature variety was sampled. Massachusetts samples were obtained from eight cultivars in 1975 and from Early Black in 1976. Sampling data were pooled because of lack of differences among cultivars.
[b] Other commonly isolated pathogens included *Physalospora vaccinii* (Shear) Arx et Müller and *Sporonema oxycocci* Shear. Some totals exceeded 100% because more than one pathogen was isolated from some fruit.

Table 2. Incidence of *Phyllosticta vaccinii* and *Botryosphaeria vaccinii* in cranberry fruit in storage

Storage temperature (C)	Sample source[a]	Year	No. of fruit decayed	Percentage of sample decayed by: *P. vaccinii*	*B. vaccinii*	Other fungi[b]
24	New Jersey[c]	1976	75	0	27	73
	Massachusetts[c]	1975	107	0	35	65
	Massachusetts[c]	1976	207	0	80	20
	Massachusetts	1976	127	0	50	50
	Wisconsin	1975	124	0	55	45
	Wisconsin	1976	228	0	88	12
	Washington	1976	118	0	76	24
10	New Jersey[c]	1976	10	0	0	100
	Massachusetts[c]	1975	311	0	1	99
	Massachusetts[c]	1976	18	0	11	89
	Massachusetts	1976	53	0	6	94
	Wisconsin	1976	22	0	5	95
	Washington	1976	71	0	1	99
4	New Jersey[c]	1976	2	0	0	100
	Massachusetts[c]	1975	214	0	0	100
	Massachusetts[c]	1976	4	0	0	100
	Massachusetts	1976	31	0	0	100
	Wisconsin	1976	26	0	0	100
	Washington	1976	22	0	5	95

[a] New Jersey and Massachusetts samples were obtained from the cultivar Early Black, Wisconsin samples from Searles, and Washington samples from McFarlin.
[b] Other fungi isolated were predominantly those previously associated with postharvest decays at the respective storage temperatures, such as *Physalospora, Diaporthe, Sporonema, Glomerella, Godronia,* and *Ceuthospora.*
[c] Plots treated with protectant fungicides.

Table 3. Incidence of *Phyllosticta vaccinii* and *Botryosphaeria vaccinii* in cranberry leaves

Sample source[a]	Sample size	Percentage of sample with: *P. vaccinii*	*B. vaccinii*	Other fungi[b]
New Jersey				
Leaf spot symptoms[c]	100	56	8	68
No symptoms	50	3	54	30
Massachusetts	125	14	42	35
Wisconsin	100	0	39	55
Washington	100	0	21	81

[a] New Jersey and Massachusetts samples were obtained from the cultivar Early Black, Wisconsin samples from Searles, and Washington samples from McFarlin.
[b] *Physalospora vaccinii* was also commonly isolated from asymptomatic leaves. Commonly occurring saprophytes were often isolated as contaminants from necrotic leaf tissue.
[c] Leaf spot symptoms were noted only on leaves collected in New Jersey.

Table 4. Percentage of inoculated cranberry fruit (cv. Searles) with decay symptoms and fruit speckle at two postinfection temperatures[a]

Species isolated	Postinoculation temperature (C)	Decay symptoms/ reisolation success (%)	Fruit speckle/ reisolation success (%)
Phyllosticta vaccinii	20	9/100	0/0
	28	63/91	0/0
Botryosphaeria vaccinii	20	6/0	68/97
	28	41/21	55/100

[a] Inoculated plants were placed in darkened dew chamber at 24 C for 72 hr. Each treatment of 15 pots of plants contained 20–44 fruit.

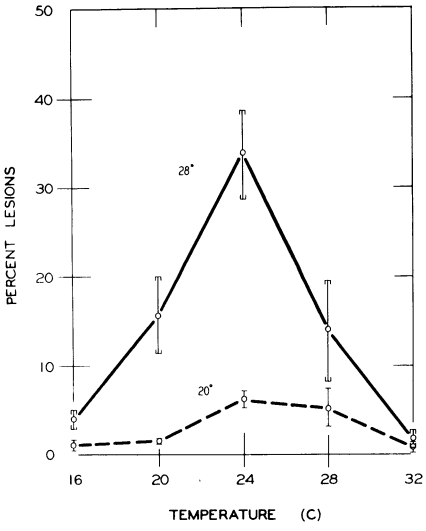

Fig. 1. Percentage of total leaves with leaf spot symptoms after inoculation with *Phyllosticta vaccinii* (isolate S6) and 72 hr of incubation at five dew chamber temperatures followed by 5 wk of growth in 20 C (dashed line) or 28 C (solid line) growth chambers. Each treatment was replicated three times. Vertical bars represent standard error.

did form developed slowly and were somewhat restricted in size.

Pathogenicity to fruit. Small, watery lesions developed on fruit inoculated with either *B. vaccinii* or *P. vaccinii* and incubated in growth chambers (Table 4). Postinfection disease development was temperature-dependent, with disease incidence seven times higher in plants maintained at 28 C than in those maintained at 20 C. Decayed tissue from fruit inoculated with *B. vaccinii* was often contaminated with common saprophytes, whereas fruit infected wtih *P. vaccinii* was not. Consequently, *P. vaccinii* was readily recovered from symptomatic fruit tissue but recovery of *B. vaccinii* from developing lesions was more difficult..

In some cases after 3 wk, superficial spots 1–2 mm in diameter were also noted on the epidermis of fruit inoculated with *B. vaccinii*. The small, pale flecks appeared in ripening fruit and did not increase in size significantly as the fruit matured. No comparable symptoms were noted on fruit inoculated with *P. vaccinii* or control plants. Postinfection symptom development was not temperature-dependent (Table 4), although symptoms were more pronounced at 20 C owing to increased fruit ripening. *B. vaccinii* was readily recovered from inoculated fruit with speckle symptoms.

DISCUSSION

P. vaccinii and *B. vaccinii* differed substantially in pathogenicity to cranberry. *P. vaccinii* appears to be the causal agent of the preharvest fruit decay and leaf spot in New Jersey and Massachusetts previously attributed to *B. vaccinii* (6,8). Isolations from field samples suggest that *P. vaccinii* is one of the primary pathogens responsible for preharvest fruit decay in New Jersey and Massachusetts; its pathogenicity to cranberry leaves and fruit was confirmed in growth chamber tests. The low incidence of leaf spot and the high incidence of fruit rot typically observed in growth chamber tests correlate well with field observations in New Jersey and previous reports in the literature (6,7). *P. vaccinii* is also apparently responsible for a previously undescribed shoot blight in New Jersey. Shear (6) reported that severely infected plants could be killed but did not describe the development of stem lesions.

P. vaccinii was not recovered from plant materials from Wisconsin and Washington, where preharvest fruit decay and leaf spot are rarely reported. The factors responsible for the apparent exclusion of *P. vaccinii* from Wisconsin and the Northwest are unknown. *P. vaccinii* has been introduced into Wisconsin in planting stock but apparently does not persist (D. M. Boone, *unpublished*). The high optimum radial growth temperature of *P. vaccinii* in culture (12) and the higher disease incidence observed on leaves and fruit after a 28-C postinfection incubation tend to support the conclusions of Stevens (9) and Shear et al (8) that disease incidence correlates with high mean summer temperatures. The observed temperature differences are insufficient to entirely rule out the possible presence of *P. vaccinii* in the other growing regions, however.

B. vaccinii appears to be responsible for postharvest fruit decay in storage and fruit speckle. It is universally distributed, as previously reported (2,8).

Although *B. vaccinii* decayed fruit in growth chamber tests, the data from naturally infected fruit samples indicate that *B. vaccinii* may not be an important contributor to cranberry fruit decay in commercial beds. Fruit grown in controlled environments takes a longer period to develop and may be subjected to stresses not normally encountered in the field, possibly influencing susceptibility. The low recovery of *B. vaccinii* from decayed tissues in growth chamber tests and the presence of contaminating organisms may reflect a low level of virulence under normal circumstances.

The results in the growth chamber tests indicate that *B. vaccinii* is a causal agent of cranberry fruit speckle. Others (2,4) have previously implicated *B. vaccinii*, but this is the first time that controlled inoculations have demonstrated the relationship. It is likely, however, that other fungi also cause fruit speckle. *Gibbera* species, causing localized infections, also can induce a similar response (2). Cranberry fruit often react to localized injuries with changes in pigmentation.

B. vaccinii is apparently able to establish latent infections on cranberry leaves and fruit, as previously reported (1,3,4,6). Fruit naturally infected by *B. vaccinii* rarely decay before harvest and storage, although infection can take place early in the growing season (1,3,10). Our studies suggest that *B. vaccinii* typically causes a superficial, dormant infection of the fruit epidermis until harvest, when active growth resumes during postharvest ripening under warm storage conditions. *B. vaccinii* was also consistently isolated from asymptomatic leaves, as reported in previous studies (3,6), and no leaf spot symptoms developed in growth chamber tests. Latent leaf infections cause no visible injury, although previous authors (3,6) suggest that infected leaves may drop prematurely.

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