Postharvest Fungi of Lowbush Blueberry Fruit

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ABSTRACT

Lambert, D. H. 1990. Postharvest fungi of lowbush blueberry fruit. Plant Dis. 74: 285-287.

The major fungi isolated from 50 processor or market samples of lowbush blueberry fruit were *Botrytis cinerea* (3.7%), *Glomerella cingulata* (3.0%), *Gloeosporium minus* (2.5%), *Alternaria* spp. (1.0%), and *Penicillium* spp. (0.8%). *Gloeosporium*, an incitant of leaf spots, stem cankers, and blossom-end rot on highbush blueberries, has not previously been reported as a disease of lowbush blueberry. The incidence of *Glomerella* and *Alternaria*, but not of *Botrytis* and *Gloeosporium*, was significantly higher in fields pruned by mowing than in those pruned by burning. Heat-tolerant fungi isolated included *Eupenicillium lapidosum*, another sclerotial *Eupenicillium* sp., *Talaromyces striatus*, an isolate resembling *Humicola* sp., and two unidentified species.

Lowbush blueberry (*Vaccinium angustifolium* Aiton) is a major crop in Maine and the eastern provinces of Canada. Production of highbush blueberry (*V. corymbosum* L.) is concentrated in Michigan, New Jersey, North Carolina, and the Pacific Northwest. Lowbush plants establish naturally, growing as a ground cover of genetically diverse clones. Fields are usually managed on a 2-yr cycle, with a single year of fruit production followed by mowing or burning and a year of vegetative growth (3). These cultural, genetic, and geographic differences suggest that the diseases of the two species differ, if only in relative severity. In Maine, where half the lowbush crop is produced, only mummy berry disease, caused by *Monilinia vaccinii-corymbosi* (Reade) Honey, and Botrytis blossom blight warrant control measures. Such measures are often not used if history of these diseases or current weather suggests that they will be unlikely or if the grower prefers not to use pesticides in the crop year. Fungicides are rarely applied after late bloom and do not prevent infection of developing or mature fruit.

Because 98% of the lowbush crop is immediately frozen for sale or for subsequent canning, little attention has been given to postharvest diseases. Heat-tolerant fungi have, however, been recovered from canned blueberries (12,16). With increased interest in fresh marketing and a desire to maximize quality of processed berries, a study of such fungi was undertaken to answer several questions: 1) What are the important fungi present in or on lowbush blueberry and does their incidence differ from that of highbush berries? 2) Does burning reduce the incidence of disease organisms? 3) Which heat-tolerant fungi are most commonly associated with the crop?

Maine Agricultural Experiment Station external publication No. 1416.

Accepted for publication 30 October 1989 (submitted for electronic processing).

MATERIALS AND METHODS

Fruit survey. In 1988, 50 fruit samples were obtained over a period of 3 wk from three major blueberry processors and from several roadside stands and supermarkets. Processors' berries were taken from boxes received from the field within the previous day. Half of each cooled sample was rinsed for 2 min in 0.5% sodium hypochlorite with swirling. Surface-disinfested and nondisinfested subsamples of 216 berries each were placed in moist chambers (cake pans with plastic lids containing wet paper towels) on 0.6-cm mesh wire screens and incubated at room temperature for 3 wk. Infected fruit were removed two times per week, and filamentous fungi growing from them were identified. In 1989, six samples of highbush blueberry fruit from three New Jersey growers were obtained in local markets, and 300 berries per sample were incubated, without rinsing, as above. Infected berries were removed daily, identified, and recorded. This small survey was designed to recover highbush fruit with blossom-end rot, which could be compared to lowbush fruit infected with *Gloeosporium minus* Shear.

Effects of burning. In 1989, fruit samples were collected at two research field sites in eastern Maine. These sites (6) were designed as split plots with pruning treatment either by mowing or by burning. At site 1, 36 samples were taken from each of the two adjacent treatments. At site 2, 30 samples were taken from each treatment. One hundred berries per sample were placed without rinsing in moist chambers and incubated at room temperature for 3 wk. During this period, infected berries were recorded and removed two times each week. Data for *Botrytis, Glomerella, Alternaria*, and *Gloeosporium* were evaluated by regression analysis with site and pruning treatment as variables to determine if the incidence of each disease was affected by the method of pruning.

Identification of heat-tolerant fungi. Strains of heat-tolerant fungi were isolated from the 50 fruit samples obtained in 1988 and from additional berry samples. The times and temperatures selected were similar to those used during canning. After being frozen at −20 C for 6 mo, 25-g samples were thawed in sterile 50-ml centrifuge tubes to which 25 ml of hot (80 C) sterile water was added. These were placed in an 82–83 C water bath and incubated for 20 min after the samples had come to 81 C (approximately 30 min total). The tubes were inspected for fungal growth over a 1-mo period, and fungi that survived the heat treatment were isolated. In addition, isolates of *Penicillium* spp. that formed sclerotia or ascocarps were saved from the fresh fruit survey.

RESULTS AND DISCUSSION

Fruit survey. The frequencies of major fruit-inhabiting fungi in 1988 are listed in Table 1. Surface disinfestation had no consistent effect on frequency of isolations, and therefore results for disinfested and nondisinfested berries were combined. Incidence of fruit-infecting fungi in samples of lowbush blueberry fruit in 1988 was highest for *Botrytis cinerea* Pers.:Fr., *Glomerella cingulata* Spauld. & Schrenk, *G. minus*, and *Alternaria* spp. *Botrytis* causes a blossom blight as well as a fruit rot (3,9,11). *Glomerella cingulata* (= *Colletotrichum gloeosporoides*) causes anthracnose or ripe rot, affecting primarily blossoms and ripe fruit (14). *G. minus* is the major leaf spot fungus of highbush blueberries in the Southeast (7,9,15). *Alternaria* is considered the most serious of highbush blueberry pathogens (8,9,11).

The relative frequency of these fungi was similar to that reported in a three-state survey of highbush fruit (2), in which incidences were *Botrytis* 3.2%, *Glomerella* 2.6%, and *Alternaria* 2.0%. *Gloeosporium* (2.5% incidence in our survey) was not reported in the highbush survey, but blossom-end rot was reported in North Carolina (2.8%), New Jersey (0.5%), and Michigan (0.8%). This anomaly and the high incidence of blossom-end rot in North Carolina (where *Gloeosporium* predominates)

suggests that the causal organism of this rot is actually *Gloeosporium*. Our subsequent (1989) sampling of highbush fruit from New Jersey detected a 3.3% incidence of *G. minus*, with three of six samples affected. In contrast to *Glomerella, Gloeosporium* acervuli were centered around the calyx (blossom end), fitting the common disease name. The size of spores recovered directly from the New Jersey fruit (5–6 × 9–11 μm) was similar but not identical to that previously reported for *G. minus* (3–4 × 6–9 μm) (7,9) and to that produced by lowbush fruit fungi (3–4 × 7–10 μm). The colony color and morphology of cultures from lowbush and highbush fruit were similar. No other unidentified acervular fungi were found in the New Jersey samples, strongly implying that blossom-end rot is the fruit symptom of *G. minus*. When spores from a lowbush isolate were used to inoculate the highbush cultivar Jersey, typical symptoms were obtained. Damaged leaves developed large lesions with adjoining vascular discoloration, nondamaged leaves became crinkled with red flecks, and cankers with acervuli developed around fresh leaf scars. *G. minus* has not been previously reported on lowbush blueberry. It has been reported on fruit of highbush blueberry (7) and cranberry (*V. macrocarpon* Aiton) (4).

In addition to the major fruit-rotting fungi listed above, *Penicillium* (at least six species), *Mucor, Aspergillus*, and several unidentified fungi were obtained less frequently in the 1988 survey.

Effects of burning. The relative incidence of major fruit-inhabiting fungi in fields pruned by mowing or by burning is given in Table 2. Isolations of *Glomerella* and *Alternaria* were significantly but not substantially higher in the mowed plots, implying that periodic burning reduces inoculum levels of these organisms. This is, presumably, a residual effect from the year prior to fruit production, since both treatments accumulate inoculum in dead leaves and winter-killed stems between the vegetative and fruit-bearing seasons. At site 1, where the pruning trials had been maintained for 12 yr, mummy berry disease (*M. vaccinii-corymbosi*) was 90-fold higher in the mowed treatment than in the burned treatment (6). At site 2 (trials maintained for 4 yr), the treatment differential for the disease was only sixfold. This compounding of disease with time was not evident for *Glomerella* and *Alternaria*. Isolations of *Botrytis* were somewhat more frequent in mowed treatments, but the difference was not statistically significant. *Gloeosporium* was recovered only twice at site 1. At site 2, nearly all isolates were from the burned treatment. Although the fungus was widely distributed in this treatment (12 of 30 samples), lack of a rational explanation and of confirming data from site 1 advise caution in interpretation of the results.

Heat-tolerant strains. *Eupenicillium lapidosum* Stolk & Scott, or a closely related species, was recovered from several samples of heat-treated berries and was also the most common anamorph of *Penicillium* isolated in the 1988 fruit survey. These isolates differed from the species description (10) only in their faster growth on malt extract agar at 25 C and on Czapek-yeast agar at 37 C. A second sclerotial *Eupenicillium* species was recovered from heat-treated fruit. This one resembled the related species *E. brefeldiamum* (B. Dodge) Stolk & Scott, *E. levitum* (Raper & Fennell) Stolk & Scott, and *E. ehrlichii* (Klebahn) Stolk & Scott (10). Additional isolates were closely related or identical to *Talaromyces striatus* (Raper & Fennell) Stolk & Sampson. A fourth type of heat-resistant isolate had a *Penicillium*-like anamorph but has not been further identified. None of the identified species is known to produce mycotoxins (10). An additional isolate produced aleuriospores similar but not identical to those of the described species of *Humicola* and *Thermomyces* (1). This fungus was slow-growing and golden-olive on potato-dextrose agar, was nonthermophilic, and did not degrade microcrystaline cellulose. The aleuriospores were 5–6 μm in diameter, had a single conical or goblet-shaped basal cell, and were spiny when mature, in contrast to those of *Humicola* and *Thermomyces*.

E. lapidosum and *T. striatus* were reported in 1941 (12,16) from canned blueberries. The other isolates reported here (excepting the single *Penicillium*-like isolate) also produce thick-walled structures. It is not known whether such resistant spores, sclerotia, or ascocarps develop in or on berries before harvest or whether fruit is contaminated by inoculum produced in the soil or on dead plant matter. *Byssochlamys* spp. (5) were not isolated, although these heat-resistant species are common on other fruit in the Northeast (13).

Table 1. Percent incidence of fruit-infecting fungi in 50 samples of Maine lowbush blueberry fruit in 1988[a]

Species	Average incidence (%)	Number of samples in incidence categories				
		0%	<1%	1-5%	5-10%	>10%
Botrytis cinerea	3.7	2	15	19	12	2
Glomerella cingulata	3.0	11	7	21	7	4
Gloeosporium minus	2.5	9	11	21	9	0
Alternaria spp.	1.0	7	26	17	0	0
Penicillium spp.	0.8	26	18	4	1	1
Trichoderma spp.	0.1	39	10	1	0	0

[a]Fruit were incubated on 0.6-cm grid mesh in moist chambers (enclosed cake pans) at room temperature for 3 wk; infected fruit were removed and recorded biweekly. Values are the means of 50 samples of 432 berries.

Table 2. Percent incidence of major fungi infecting Maine lowbush blueberry fruit at two sites in 1989 as affected by pruning treatment (mowing vs. burning)[a]

Species	Site 1		Site 2		Treatment significance[b]
	Mowed	Burned	Mowed	Burned	
Botrytis cinerea	3.9	3.1	5.5	3.9	NS
Glomerella cingulata	5.1	2.9	13.3	4.4	0.01
Alternaria spp.	1.5	0.9	1.2	0.4	0.05
Gloeosporium minus	0.0	0.1	0.1	1.3	0.01

[a]Nondisinfested fruit were incubated on 0.6-cm grid mesh in moist chambers (enclosed cake pans) at room temperature for 2 wk; infected fruit were removed and recorded biweekly. Values are the means of 36 samples of 100 berries from site 1 and 30 samples of 100 berries from site 2.

[b]Probabilities of differences according to pruning treatment. Treatment effects on *Botrytis, Glomerella,* and *Alternaria* were evaluated as factors in an analysis of variance. For *Gloeosporium,* only site 2 data were analyzed, by *t* test. NS = not significant.

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