Effect of Wounding on Incidence of Black Rot of Cranberry in Wisconsin

M. R. SCHWARZ, Research Assistant, and D. M. BOONE, Professor, Department of Plant Pathology, University of Wisconsin, Madison 53706

ABSTRACT

Schwarz, M. R., and Boone, D. M. 1985. Effect of wounding on incidence of black rot of cranberry in Wisconsin. Plant Disease 69:225-227.

Laboratory trials showed that *Ceuthospora lunata* was primarily a wound-invading organism. Pycnidiospores germinated at a higher frequency on fruits that were wounded by puncturing the epidermis than on unwounded fruits kept at temperatures between 8 and 24 C. Black rot developed in more than 92% of cranberries wounded and dipped in a spore suspension. Control or bruised cranberries without a punctured epidermis showed little or no black rot. Cranberry leaf debris containing mature pycnidia of *C. lunata* was a principal source of black rot inoculum during the growing season and during wet-rake harvesting in October. Harvest water was an important medium for dissemination of *C. lunata* spores. Viable *C. lunata* spores at concentrations as high as 119/ml were found at harvest in flood waters used for wet-raking cranberries. Black rot developed in more than 46% of wounded cranberries immersed in harvest water samples, whereas no black rot developed in comparable cranberries immersed in heat-sterilized harvest water.

Black rot, a storage disorder of fresh-market cranberry (*Vaccinium macrocarpon* Ait.) fruit caused by *Ceuthospora lunata* Shear, is reported from all major commercial cranberry-growing regions (5,11). Two cultural strains of *C. lunata* are recognized. One produces gray-brown cottony mycelium (LT strain) and the other produces gray-black, appressed mycelium (DK strain) in culture (4). *Strasseria oxycocci* Shear has also been identified as an incitant of black rot in New Jersey but not in Wisconsin (8).

Most cranberry pathogens are speculated to infect fruit early in their development and then rot the berries later during storage (11,12,15,16). Bruises predispose cranberries to other storage decays but no conclusions can be drawn regarding *C. lunata* (6). Previous investigators found that wet-raking, a method of harvesting cranberries in flooded beds, resulted in inferior keeping quality and more injuries to fruit than dry-raking (2,13). This was related to the greater frequency of black rot in wet-raked cranberries than in dry-raked ones (1,14). *C. lunata* may infect fruit through wounds made before or during harvest and by growing into fruit tissue from attached necrotic or senescent sepals (4).

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706, by USDA-SEA Hatch formula funds (Project 6011), and by funds supplied by Wisconsin cranberry growers through a Marketing Order for Cranberries administered by the Wisconsin Department of Agriculture, Trade, and Consumer Protection.

Accepted for publication 1 September 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Mature pycnidia of *C. lunata* were found in dead and dying cranberry leaves collected just before harvest in September (9,10).

Wet-raking is now used extensively to harvest cranberries in Wisconsin and Washington (2) and is gaining acceptance on East Coast bogs (4,14). This study was conducted to determine whether water used during wet-raking in Wisconsin was a medium for dissemination of *C. lunata* pycnidiospores, to assess the principal mode of infection, and to identify a source of black rot inoculum. A preliminary report of a portion of this work has been published (7).

MATERIALS AND METHODS

Samples of cast cranberry leaves and previous season's fruit were collected periodically during the 1977, 1978, and 1979 growing seasons from debris piles and from the ground underneath living vines at two commercial cranberry marshes (Russel Rezin Cranberry Co., Warrens, WI, and Gottschalk Cranberry Co., Inc., Wisconsin Rapids, WI). Samples were stored at 4 C in plastic bags for less than 2 wk before use. Debris was examined under a dissecting microscope until about 30 leaves and fruit were found containing pycnidia like those of *C. lunata* or until about 200 leaves and fruits had been screened. Selected debris was then placed in a sterile moist chamber for 24–48 hr to induce sporulation. Ordinarily, spores or surgically removed pycnidia were mounted in water or lactophenol cotton blue for examination at ×320 by phase-contrast microscopy. Single-spored cultures were occasionally prepared by suspending spores in a sterile water droplet and streaking them onto a water-agar (WA) plate. The identity and maturity of each fruiting structure examined were recorded.

Ripe, fungicide-free cranberries (cultivar Searles) were collected from the Gottschalk Cranberry Co. and stored in paper bags at 4 C for 1 mo before use. Fruits were then surface-sterilized for 5 min in 1% NaOCl plus 0.05% Tween 80 and rinsed several times in heat-sterilized water, then either left sound, bruised by indenting the epidermis with a blunt instrument, or wounded by puncturing the epidermis with four pins inserted in a cork in a 5-mm-square configuration so that the points protruded 4 mm. Berries were inoculated by immersing them for 5 min in a spore suspension or in sterile water. The spore suspension, composed of an equal mixture of spores from several DK and LT single-spored isolates of *C. lunata*, was adjusted to 1×10^5 spores per milliliter with a hemacytometer. One hundred berries were used for each treatment and each treatment was replicated three times. After inoculation, the berrries were incubated for 72 hr at 20 C, then stored in paper bags at 4 C. After 5 wk of storage, incidence of black rot in each treatment was determined.

A comparison was made of the frequency of spore germination on wounded and unwounded fruits. Cranberries were surface-sterilized as before, placed in sterile moist chambers, and half of them were wounded using a sterile dissecting needle to puncture three holes 2 mm apart on their exposed surfaces. The remaining cranberries were not wounded. All fruits were sprayed until wet with a suspension containing LT and DK spores (1×10^6/ml). The berries were then incubated for 48 hr at four-degree intervals from 4 to 36 C. Treatments at each temperature were replicated three times. Each berry was considered a replicate. After incubation, a rectangle of epidermis (0.5 × 1.0 cm) incorporating wound holes, if present, was surgically removed from each fruit and mounted in lactophenol cotton blue. At least 100 spores were examined at ×128 or 320 using bright-field microscopy and considered germinated if the germ tube was longer than the spore.

To determine the concentration of *C. lunata* spores in harvest water, one to three 1-L samples of water used in wet-rake harvesting were collected from several cranberry marshes (DuBay Cranberry Co., Stevens Point, WI, Gottschalk Cranberry Co., Jacob Searles Cranberry Co., Stevens Point, WI, Russel Rezin Cranberry Co. and Tomahawk Cranberry Co., Tomahawk, WI), stored at 8 C, and tested within 14

days. Samples were then agitated briefly and filtered through two layers of cheesecloth to remove large debris. Samples collected in 1977, 1978, and 1979 were filtered through Whatman No. 1 filter paper or 15-μm nylon mesh, whereas those collected in 1980 were filtered through cheesecloth only. Ordinarily, three to five aliquots (0.5 ml/plate) of each undiluted harvest water sample were pipetted on plates of WA or WA plus 0.125% lactic acid and spread evenly with a sterile glass rod. After 2–7 days of incubation at room temperature (RT) (about 21 C), developing fungal colonies were observed at ×30 or 60. During the first 3 yr of processing samples, all fungal colonies were aseptically removed from each plate, subcultured at RT in separate potato-dextrose agar (PDA) slants, and identified. In 1980, direct counts of *C. lunata* colonies were made from the plates.

To test whether inoculum present in harvest water could cause black rot, dry-raked cranberries (cultivar Searles) were surface-sterilized, wounded, and immersed for 72 hr at 8 C in unsterile harvest water, heat-sterilized harvest water, or heat-sterilized distilled water. Harvest water was obtained from the Gottschalk marsh on 16 October 1979. One hundred berries were used for each treatment and all treatments were replicated three times. The berries were then air-dried and stored at 4 C in paper bags. After 2 mo, berries with black rot were counted and isolations made from 60 black-rotted fruits.

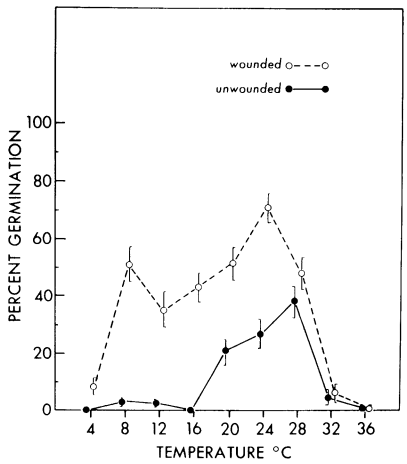

Fig. 1. Percent germination of *Ceuthospora lunata* spores on the epidermis of wounded and unwounded cranberries at nine constant temperatures. Vertical lines represent confidence limits ($P = 0.05$).

RESULTS

C. lunata pycnidia were found occasionally in detached necrotic leaves that were not greatly decomposed. No fruiting structures of the fungus were found in previous season's fruit. Fruiting structures were found most often in leaves coming from moist environments, but this was highly variable. The pycnidia were usually scattered on the abaxial leaf surface. Both *C. lunata* strains produced pycnidia in leaves. Immature pycnidia, containing conidiogenous materials but no mature conidia, were found in leaf debris sampled during May, June, and July. Mature pycnidia were observed in necrotic leaves in May, June, July, September, and October and were present in leaves found floating in flooded cranberry beds during harvest in October. No pycnidia were found in badly decomposed leaves sampled once during August. Old fruiting bodies, containing a small number of conidia but devoid of most conidiogenous materials, were only found during June and October.

After 48 hr, spores on wounded fruits had germinated at a higher frequency than spores on unwounded fruits when both were incubated at temperatures of 8–24 C. Spores germinated best (71.3%) on wounded fruits kept at 24 C (Fig. 1). At 4 or 28 C, there was no apparent difference between germination of spores on wounded and unwounded fruits. Only a small percentage of spores germinated at 32 or 36 C. Some germ tubes grew in the direction of and entered wound holes, but this was not quantified. No direct penetration by the fungus through intact epidermis was seen.

Cranberries that were wounded and then inoculated by dipping them in a suspension of *C. lunata* spores developed a significantly higher incidence of black rot than bruised or sound fruits inoculated in a comparable manner (Table 1). Incidence of the disease in wounded cranberries inoculated with a spore suspension was significantly higher than incidence of black rot in wounded, bruised, and sound cranberries dipped in sterile water and bruised and sound berries inoculated with a spore suspension. All black rot that developed in treatments 2, 3, and 5 was associated with small wounds that were probably present on the fruits before the experiment was performed.

Concentrations of *C. lunata* spores detected in harvest water ranged from a low of seven spores per milliliter at the DuBay marsh to a high of 119 spores per milliliter at the Duckert marsh (Table 2). All harvest-water samples contained some *C. lunata* spores. Either DK or LT fungal colonies developed from spores found together in samples taken from the Russel Rezin, Tomahawk, and Jacob Searles marshes. Only the LT strain was detected in samples collected from the DuBay marsh on 14 October 1977 and from the Gottschalk marsh on 16 October 1979 and 2 October 1980, even though both strains were previously isolated from cranberry material taken from the latter marsh.

A sufficient number of *C. lunata* spores were suspended in harvest water (found to contain at least 43 spores per milliliter) to cause a significant increase in the incidence of black rot when wounded cranberries were dipped in the water. Wounded cranberries immersed in sterilized harvest water or distilled water did not develop black rot (Table 3). Isolations made from cranberries that developed black rot revealed that 78% of the disease was caused by the LT strain but 22% was caused by the DK strain, even though spores of this strain had not been detected in this water previously.

DISCUSSION

C. lunata probably overwinters in leaf debris as mature pycnidia and immature

Table 1. Effect of wounding or bruising of cranberry fruits on the incidence of black rot in berries inoculated by dipping them in a spore suspension of *Ceuthospora lunata* spores

Berry treatment	Inoculum	Incidence of black rot (%)[a]
Wounded	Spore suspension	92.6 ± 4.5
	Sterile water	3.0 ± 1.0
Bruised	Spore suspension	2.0 ± 1.0
	Sterile water	0
Sound	Spore suspension	2.0 ± 1.7
	Sterile water	0

[a] Mean of three replicates followed by the standard error of the mean. Using orthogonal contrasts of treatments, wounded with spore suspension versus all other treatments was significantly different ($P < 0.001$, arc sine transformation of percentage).

Table 2. Concentration of *Ceuthospora lunata* spores in harvest water taken from flooded beds in four commercial cranberry marshes

Marsh	Sampling date	Mean spore concentration (no./ml)
DuBay	14 Oct. 1977	70 NR[a]
Russel Rezin	10 Oct. 1978	40 NR
Gottschalk	16 Oct. 1979	43 ± 5.8
Gottschalk	2 Oct. 1980	12 ± 4.3
Jacob Searles	3 Oct. 1980	119 ± 25.0
Tomahawk	7 Oct. 1980	80 ± 7.3
DuBay	9 Oct. 1980	7 ± 4.2

[a] Standard error is given only for mean values. NR = samples not replicated.

Table 3. Incidence of black rot in wounded cranberries that were immersed in unsterile or heat-sterilized harvest water or sterile distilled water

Treatment	Incidence of black rot (%)
Unsterile harvest water	46.3 b[z]
Sterile harvest water	0.0 a
Sterile distilled water	0.0 a

[z]Means followed by different letters are significantly different ($P = 0.01$) according to Tukey's multiple range test.

pycnidia that mature during the early months of the growing season because these structures are found during early May, which is only 1–2 wk after winter ice has melted from Wisconsin cranberry marshes. Because mature pycnidia are produced in infected fruits in the laboratory, they might have been found in diseased fruits in the field if a larger sample had been examined. Pycnidiospores are probably liberated during moist field conditions throughout the growing season, because mature pycnidia in leaf debris swell and extrude spores within 24–48 hr in moist chambers. These spores may germinate to colonize senescent and necrotic cranberry tissues, as proposed previously (4).

Cranberry fruits are apparently only infected by *C. lunata* through wounds. In all cases during this investigation, ripe and unripe black-rotted fruits from Wisconsin always contained small wounds where the epidermis was broken. Although Graham et al (6) found that bruising predisposes cranberries to storage decays, such is not the case with black rot caused by *C. lunata* where the incidence of this disease is significantly greater only when cranberries are wounded. Friend (4) suggested that *C. lunata* infects fruit by growing into sound fruit tissue from attached sepals, but no evidence for this or other latent infection sites was found.

Because berries are apparently infected only through wounds, further investigation might reveal cranberry varieties that are less susceptible to black rot because their fruits have a thick epidermis that resists mechanical injury or their fruits detach from the pedicels during harvest without tearing the epidermis.

Spores carried in harvest water are probably the principal source of black rot infections, as suggested by Friend (4). Fruits wounded before or during the wet-raking process are probably infected while floating in harvest water that contains concentrations of *C. lunata* pycnidiospores capable of causing disease. The longer fruits are held in harvest water, the greater the chances of wounded fruits developing the disease, as shown by Stretch and Ceponis (14). Most berries that begin to show black rot symptoms within the first months of storage were probably infected with *C. lunata* during harvest in September and October. The increase in the incidence of black rot in Wisconsin from that reported by Shear et al (11), as pointed out by Friend (4), can be attributed in part to the increased popularity of wet-raking in Wisconsin between the 1920s (13) and today (3). Wet-raking may also be partly responsible for reported increases of black rot in areas on the East Coast, where wet-raking is becoming widespread (4,14). These conclusions, however, are speculative until more work is done to determine the significance of *S. oxycocci* as an incitant of black rot there.

Both the LT strain and the DK strain of *C. lunata* apparently have similar disease cycles, because there were only minor differences detected during this investigation in the way the strains responded to various tests. LT strain spores may have been selected from cultured harvest water at a higher frequency than DK strain spores because preliminary investigations indicated that pycnidiospores from LT strains germinate faster than those from DK strains.

Preharvest protectant-eradicant fungicides are probably ineffective for black rot control because they cannot be applied late enough in the growing season to provide adequate protection for berries that are infected by *C. lunata* immediately before or during harvest. In addition, wet-raking probably helps wash off fungicides as berries are agitated in harvest water.

Future work on control of black rot in fresh-market cranberries should concentrate on the development of postharvest chemical and controlled-atmosphere treatments. Harvesting methods that minimize damage to fruits, quickly remove fruits from harvest water, and rapidly dry the berries will probably reduce the amount of black rot infection. A thorough understanding of the epidemiology of *S. oxycocci* is needed before effective control measures can be developed for areas where this fungus is also responsible for black rot (8).

LITERATURE CITED

1. Carlson, L. W. 1963. Physiology, pathogenicity, and control of fungi causing certain cranberry diseases. Diss. Abstr. 25:1331.
2. Crowley, D. J. 1954. Cranberry growing in Washington. Wash. Agric. Exp. Stn. Bull. 554. 29 pp.
3. Dana, M. N., and Klingbeil, G. C. 1966. Cranberry growing in Wisconsin. Wis. Agric. Ext. Circ. 654. 39 pp.
4. Friend, R. J. 1968. Incidence and pathogenicity of fungi found on cranberry in Wisconsin. Diss. Abstr. 29:438-B.
5. Gourley, C. O., and Harrison, K. A. 1969. Observations on cranberry fruit rots in Nova Scotia 1945–1955. Can. Plant Dis. Surv. 49:22-26.
6. Graham, S. O., Patterson, M. E., and Allen, B. 1967. Bruising as a predisposing factor in the decay of stored cranberries. Phytopathology 57:497-501.
7. Schwarz, M. R., and Boone, D. M. 1981. The effect of wounding and wet raking on the incidence of black rot of cranberries in Wisconsin. (Abstr.) Phytopathology 71:253-254.
8. Schwarz, M. R., and Boone, D. M. 1983. Black rot of cranberry caused by *Strasseria oxycocci*. Plant Dis. 67:31-32.
9. Shear, C. L. 1907. New species of fungi. Bull. Torrey Bot. Club 34:305-317.
10. Shear, C. L. 1907. Cranberry diseases. U.S. Dep. Agric. Bur. Plant Ind. Bull. 110. 64 pp.
11. Shear, C. L., Stevens, N. E., and Bain, H. F. 1931. Fungous diseases of the cultivated cranberry. U.S. Dep. Agric. Tech. Bull. 258. 58 pp.
12. Stevens, N. E. 1924. Notes on cranberry fungi in Massachusetts. Phytopathology 14:101-107.
13. Stevens, N. E., and Bergman, H. F. 1921. The relation of water-raking to the keeping quality of cranberries. U.S. Dep. Agric. Bull. 960. 12 pp.
14. Stretch, A. W., and Ceponis, M. J. 1983. Influence of water immersion time and storage period on black rot development in cold-stored, water-harvested cranberries. Plant Dis. 67:21-23.
15. Wilcox, R. B. 1935. Cranberry fruit rots in New Jersey. N.J. Agric. Exp. Stn. Circ. 340. 4 pp.
16. Wilcox, R. B. 1940. Cranberry fruit rots in New Jersey. N.J. Agric. Exp. Stn. Circ. 403. 4 pp.