Relation of Floral Infection to Botrytis Blossom-End Rot of Pears in Storage

N. F. SOMMER, Postharvest Pathologist, J. R. BUCHANAN, Specialist, and R. J. FORTLAGE, Staff Research Associate, Department of Pomology, University of California, Davis 95616; and B. E. BEARDEN, Farm Advisor, University of California, Cooperative Extension, Ukiah 95482

ABSTRACT

Sommer, N. F., Buchanan, J. R., Fortlage, R. J., and Bearden, B. E. 1985. Relation of floral infection to Botrytis blossom-end rot of pears in storage. Plant Disease 69:340-343.

Stamens and styles of Bartlett pears were infected by *Botrytis cinerea* and other fungi. Retention of colonized floral fragments within the floral tube of the mature pear was associated with blossom-end rot of fruit in storage. Fruit rotting normally occurred late in the storage period, when fruits were judged to be postclimacteric. Spraying blossoms with benomyl at petal fall reduced colonization of floral parts and rot of fruits in storage. Cooling fruits to <3 C before storage at 0 C delayed senescence and provided an effective commercial control.

In California, Botrytis rot, caused by *Botrytis cinerea* Pers. ex Fr., is common in Bartlett pear (*Pyrus communis* L.) fruits stored in air at 0 C (6). Because the fungus grows at −2 to −3 C (2; N. Sommer, *unpublished*), it cannot be halted by low temperatures without freezing the fruit.

Until recent years, Botrytis rot was unimportant in the marketing of fresh Bartlett pears in California. Rot that did occur usually originated in wounds inflicted during harvesting and handling. In rare instances, infection began in the pedicel or at the blossom end (6). Starting in the early 1970s, however, Botrytis rot caused important losses in pears grown in Lake and Mendocino counties. The lesion almost always started at the blossom end and first appeared near the end of the 3-mo commercial storage period. Loss from Botrytis blossom-end rot (BBER) varied from serious to negligible, depending on the year. Although only a small fraction of the total crop was involved, economic losses were significant because of the cost of prolonged storage and the lost potential for a high selling price.

The objectives of our study were to determine the relation of fungus-colonized stamens and styles to fruit rot in storage, the possibility of controlling fruit rot in storage by sprays at blossoming, and the relation of water dumps to subsequent fruit rot in storage. Ancillary studies and observations explored the relation of fruit rot to fruits that had become senescent and the possibility of controlling rot by improved temperature management to delay the onset of senescence.

Accepted for publication 22 October 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.

MATERIALS AND METHODS

Water dumps and rot incidence. The effect of emptying fruits from bins into water on arrival at the packinghouse was tested in two facilities. Pears from six cartons of about 99 fruits each were passed through the water dump, and fruits from six similar cartons were hand-dumped onto conveyor belts. Fruits were replaced in cartons and stored at 0.6 C at the postharvest laboratory, Department of Pomology, University of California, Davis. Data were obtained after infected fruits showed well-developed rot lesions but before appreciable mycelial "nesting" had occurred.

Experimental plots. Plots to study floral colonization during fruit development and incidence of rot in storage were established in three orchards in Mendocino County. In 1977, 16 trees were provided in each of two plots and eight were provided in another. In 1978, there were 16 trees in each plot. Benomyl (0.6 g/L) was sprayed to runoff at about 95% petal fall on one-half the trees in each plot. The remaining trees served as unsprayed controls.

***Botrytis*-colonized styles and stamens.** Twenty fruits per tree were picked from the previously described plots—five from each quadrant at a height of 1–2 m. The first 20-fruit samples were taken within 15–18 days of petal fall; the last samples were picked within 5 days of the start of commercial harvest. Intermediate samples were taken between 55 and 65 days after petal fall.

Stamen and style remnants were surface-sterilized by first submerging the fruits for 1 min in 0.05% sodium hypochlorite solution containing Tween 80 (two drops per liter). The blossom end was cut from the rest of the fruit and longitudinally bisected to expose the floral tube. Submersion in 70% ethyl alcohol provided thorough wetting of stamens and styles. After removal with sterile forceps, stamens and styles were submerged for 1 min in hypochlorite solution.

All stamens and styles from a single fruit were positioned, without prior rinsing, on the surface of 15 ml of potato-dextrose agar (PDA) in petri dishes. Initial incubation was at room tempera-

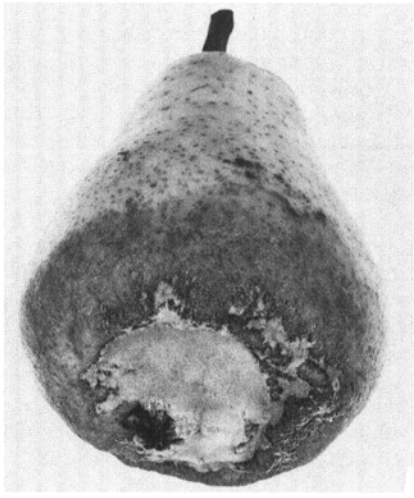

Fig. 1. Blossom-end rot of Bartlett pear with abundant sporulation of *Botrytis cinerea*.

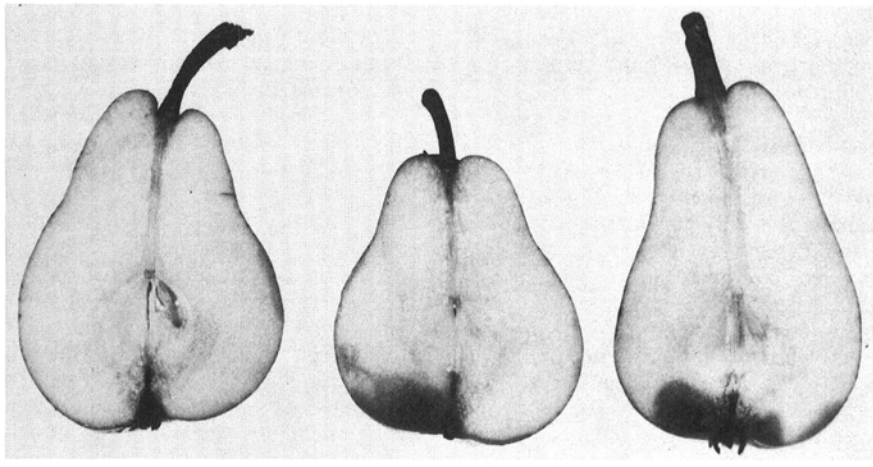

Fig. 2. Bisected Bartlett pears with lesions caused by *Botrytis cinerea* associated with the floral tube.

ture for 18–24 hr to initiate fungal growth, followed by 24–48 hr at 0 C to inactivate cold-sensitive fungi (8). Incubation was for 3–6 mo at 5 C, a temperature employed to further inhibit all fungi that do not grow at low temperatures. The presence of *B. cinerea* in cultures was detected by examination with the aid of a dissecting microscope at ×10.

Rot in storage. Samples of 30 fruits per tree were picked at the start of commercial harvest from the previously described plots to determine the incidence of rot in storage of fruits from plots in which the incidence of floral colonization by *B. cinerea* had been studied. Samples from each tree (1–2 m above the ground around the tree) were placed in vented plastic bags and transported promptly to the postharvest laboratory of the Department of Pomology. Bags of fruit were spaced on shelves at 0 C to remove field heat promptly. Fruits were subsequently placed in cartons for storage at 0.6 C. Fruits were examined at 2- or 3-wk intervals after a 3-mo storage period. Data were taken after the most advanced lesions in diseased fruits were about one-third the length of the pear but before mycelium from more than an occasional rotting fruit had infected adjacent sound fruits.

RESULTS

The first symptoms of BBER were observed at the fruit calyx (Fig. 1). Fruit rot was never seen until near the end of the storage period, when fruits were judged on the basis of a green to yellow color change to be postclimacteric. Lesions invariably adjoined the floral tube when fruits were bisected longitudinally (Fig. 2). Typically profuse aerial mycelium with or without sporulation covered the surface of the lesion. The aerial mycelium invaded adjacent sound fruits in storage, causing the formation of "nests" of rotting fruit bound together by mycelium.

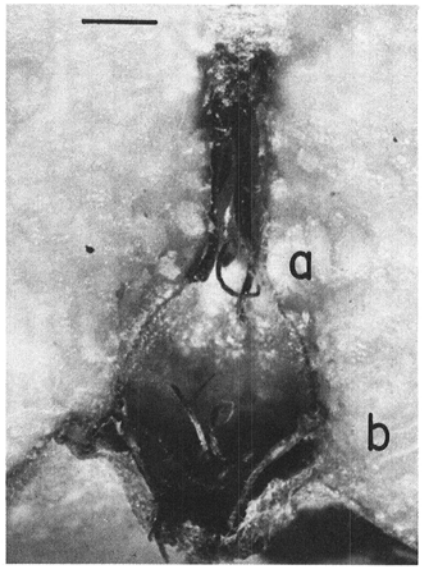

Fig. 3. Floral tube of Bartlett pear with remnants of (a) styles and (b) stamens. Bar = 25 mm.

The effects of water submersion on BBER were tested in two commercial packinghouses. In one, 33 of 415 fruits rotted in storage after wet dumping, whereas only 6 of 400 dry-dumped fruit rotted (a difference significant at $P = 0.001$ using the chi-square test). In contrast, only 5 of 534 water-dumped fruit and 1 of 471 dry-dumped fruits in a second packinghouse rotted.

Fruits retain stamens and styles or their remnants throughout the life of the fruit (Fig. 3). These dead floral parts were almost invariably colonized by fungi. Indeed, extracts of floral parts of several species have been reported to stimulate germination of *B. cinerea* (1). The most prevalent fungi were *Alternaria* spp. and *Cladosporium* spp., which were found in one or more of the stamens and styles of nearly every fruit. Also found commonly were *Stemphylium* spp., *Fusarium* spp., *Aspergillus* spp., *Penicillium* spp., *Colletotrichum* spp., and *Chaetomium* spp. The fraction of pear fruits with at least one stamen or style colonized by *B. cinerea* varied from nearly none to more than 60% (Fig. 4, Table 1).

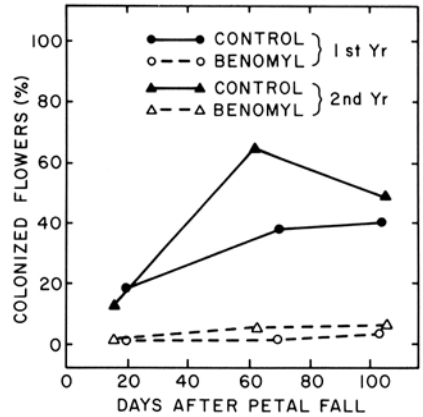

Fig. 4. Percentage of fruits with styles or stamens infected with *Botrytis cinerea* from 15–18 days after petal fall until 5 days before harvest as affected by a benomyl spray at petal fall.

In 1977 and 1978, pears from an area where BBER occurred in storage were examined (Fig. 4). Within 15–18 days of petal fall, 15–20% of the fruits had at least one stamen or style colonized by *B. cinerea*. Higher incidences at midseason and at harvest indicated that further infections occurred during fruit growth and maturation. An application of benomyl at petal fall caused the incidence of stamens and styles infected by *B. cinerea* to remain low.

In 1977, BBER was absent or scarce in fruit from two of three plots despite 30 and 24% incidence of fruits with stamens and styles colonized by *B. cinerea* at harvest in plots 1 and 3, respectively (Table 1). The remaining plot had an incidence of 61% of the fruits with infected stamens and styles at harvest, and 5% rotted in storage although no rot occurred in fruits sprayed with benomyl at petal fall. Results in 1978 showed roughly the same incidence of unsprayed fruit with infected floral parts at harvest, but rot in storage varied from 4 to 7%. Fruits sprayed at petal fall had 4.4–8.8%

incidence of *B. cinerea* in floral parts at harvest, which resulted in 1% storage rot in fruit from plots 1 and 2 but no rot in fruit from plot 3.

DISCUSSION

The possibility that water dumps increase the likelihood of BBER in storage was only partially supported by these studies. It is clear that water dumps can cause an increase in the incidence of BBER, particularly if sodium hypochlorite or another sanitizing agent is not present in the water to prevent the accumulation of viable inoculum. Perhaps the presence of water in the floral tube facilitated the spread of the fungus from colonized stamens and styles to the fruit flesh. Nevertheless, the occurrence of BBER after fruits had been dry-dumped suggested that the contaminated dump water was not necessarily essential for infection and disease development.

We believe the evidence is strong that BBER in stored pear fruits is due in large measure to infection of styles and stamens in the orchard. The evidence is as follows: 1) small rot lesions invariably adjoined the floral tube or subcalyx area, 2) *B. cinerea* colonized stamens and styles, 3) fruit rot was highest among fruits with a high incidence of infected stamens and styles, and 4) benomyl application near the time of petal fall dramatically reduced the incidence of infected stamens and styles and significantly reduced the incidence of storage rot.

Studies in Mendocino County revealed that orchards with a high incidence of *B. cinerea* in fruit can be less than a kilometer from orchards with a very low incidence. Reasons for the variability from one location to another are unknown. The theory that permanent ground cover or that nearness to vineyards might increase the incidence was not confirmed. The much earlier appearance of yellowing and of BBER in commercial storage than in our tests suggested that an important difference in temperature management existed. Temperatures in commercial storage and in our experimental facility were both held near 0 C, but we believe field heat was removed much more slowly in commercial storage rooms.

Guillou (3) studied the time to cool Bartlett pears in commercial storage rooms and compared cooling rates by use of "half-cooling" times. The half-cooling time was the period required to cool the fruit to 50% of the difference between the initial fruit temperature and the air temperature in the cooling room. A second, equal time would be required to lower the temperature to 75% of the initial difference, and a third half-cooling time would reduce fruit temperature to 87.5% of the initial difference (seven-eighths cooled).

When fruits were placed in storage rooms without prior cooling, the half-cooling time varied from 20 to 45 hr, depending primarily on spacing of the cartons on pallets (3). Thus a period between 60 and 135 hr would be required to reach the seven-eighths-cooled point. To those times must be added 4–12 hr for transportation from the orchard and for packinghouse operations before the start of cooling. Fruits in our tests, by contrast, required about 6 hr before they were placed in storage at Davis. However, the half-cooling time was only 3.5 hr, when the plastic bags containing 30 fruits each were spaced in a room at 0.6 C. Only 16.5 hr were required to reach the seven-eighths-cooled point, including the 6-hr transit to Davis.

The forced-air cooling method (5), when commercially adopted for Bartlett pears, has been shown in tests to have a half-cooling time of 2 hr, requiring only 6 hr to be seven-eighths cooled. In the 5 yr since the adoption of forced-air cooling, no BBER has been reported in storage. Similarly, no BBER has been observed in market areas in fruits that had been properly forced-air-cooled. An exception has been with a few paper-wrapped fruits where the paper prevented adequate movement of cooling air.

We believe better temperature management has delayed the climacteric and the onset of senescence. The normal disease resistance of the fruit is consequently retained longer. Evidently with this improved storage, fruits reach the consumer, ripen, and are consumed before the fungus becomes noticeably active.

Although the disease has been effectively avoided by improved temperature management, *B. cinerea* in colonized stamens and styles remains viable and potentially active. Delays, inadequate refrigeration in transport or market areas, or shipping to more distant markets involving much longer periods for transportation could cause the disease to reappear. In that event, results of this study suggest that fungicidal sprays at about the time of petal fall could prevent colonization of stamens and styles and would probably provide added fruit protection.

The percentage of pears with fruit rot was never as great as the incidence of fruits with colonized floral parts. Factors favoring growth of fungi from colonized floral remnants into susceptible fruit flesh are not known. The presence of water in the floral tube resulting from use of water dumps may increase the chances for invasion of the fruit flesh. Competing fungi colonizing the same floral part may suppress *B. cinerea*. We believe, however, that the physiological condition of the fruit plays a key role. Evidently *B. cinerea* is capable of spreading from the floral tube into the fruit flesh only after the pear has become senescent. The disease resembles, in part, quiescent infections of strawberry fruits (7), grape berries (4), and raspberry and apple (9), in which pathogenic relationships are not established until the fruit ripens. Reasons for the increased problem with this form of Botrytis rot during the 1970s, compared with previous years, were not ascertained.

Table 1. Effect of fungicide application at petal fall on the incidence of pear fruits with stamens or styles colonized by *Botrytis cinerea*, and on fruit rot in storage

Plot	Infected stamens or styles at harvest (%)[a]		Fruit rot in storage (%)[b]	
	Control	Benomyl[c]	Control	Benomyl
		1977[b]		
1	30	1.2***[d]	0.0	0 NS
2	61	2.5***	5.0	0***
3	24	1.2***	0.4	0 NS
		1978		
1	39	8.8***	7.0	1**
2	52	4.4***	6.0	1*
3	29	4.6***	4.0	0**

[a] Fruits (%) with stamens or styles colonized by *B. cinerea* at harvest. Each datum was determined from 160 fruits, except plot 3 in 1977 with 80.

[b] Fruit rot (%) in storage. Each datum was determined from 240 fruits, except plot 3 in 1977 with 120.

[c] Sprays applied 19 April 1977 and 26 April 1978.

[d] Sprayed fruits were not significantly (NS) or significantly different from unsprayed controls at * = $P = 0.05$, ** = $P = 0.01$, or *** = $P = 0.001$ as determined by the chi-square test.

LITERATURE CITED

1. Borecka, H., and Millikan, D. F. 1973. Stimulatory effect of pollen and pistillate parts of some horticultural species upon the germination of *Botrytis cinerea* spores. Phytopathology 63:1431-1432.
2. Brooks, C., and Cooley, J. S. 1917. Temperature relations of apple rot fungi. J. Agric. Res. 8:139-164.
3. Guillou, R. 1960. Coolers for fruits and vegetables. Calif. Agric. Exp. Stn. Bull. 773. 65 pp.
4. McClellan, W. D., and Hewitt, W. B. 1973. Early Botrytis rot of grapes: Time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. Phytopathology 63:1151-1157.
5. Mitchell, F. G., Guillou, R., and Parsons, R. A., 1972. Commercial cooling of fruits and vegetables. Univ. Calif. Div. Agric. Sci. Man. 43. 44 pp.
6. Pierson, C. F., Ceponis, M. J., and McColloch, L. P. 1971. Market diseases of apples, pears and quinces. U.S. Dep. Agric. Agric. Handb. 376. 112 pp.

7. Powelson, R. L. 1960. Initiation of strawberry fruit rot caused by *Botrytis cinerea*. Phytopathology 50:491-494.
8. Sommer, N. F., Matsumoto, T. T., and Fortlage, R. J. 1971. Chilling inactivation of postharvest pathogens of fruits and vegetables. Proc. Int. Cong. Refrig., 13th 3:173-176.
9. Verhoeff, K. 1980. The infection process and host-parasite interactions. Pages 153-180 in: The Biology of *Botrytis*. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, New York. 318 pp.