Occurrence and Relative Susceptibility of Apple Cultivars to Botryosphaeria Canker in Chile

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ABSTRACT

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A canker disease initially observed on the trunks of Red King Oregon apple trees was shown to be caused by *Botryosphaeria dothidea*, a fungus not previously reported on apples in Chile. Trunk symptoms were characterized by the presence of flat, sunken cankers. Initially, the surface of the bark turned orange to pink, developed a papery appearance, and sloughed away. Abundant black pycnidia extruded through the outer layer of the bark. All *B. dothidea* isolates tested caused fruit rot on apple cultivars Granny Smith, Starking Delicious, and Richared Delicious and produced cankers after 8 wk on the trunks of artificially inoculated 1-yr-old Red King Oregon apple trees. Trunk susceptibility was dependent ($P = 0.01$) on cultivar, isolate, and inoculation position. Red King Oregon was the most susceptible cultivar, followed by Red Spur and Starkrimson. Granny Smith, Granny Smith Spur, and Golden Spur were the most resistant cultivars. Differences in virulence among fungal isolates were observed. At present, Botryosphaeria canker of apple is considered of minor importance but is a potential threat to the Chilean apple industry.

An unusual canker disease of the trunk of apple (*Malus pumila* Miller) appeared in a 2-yr-old orchard near Curicó, Chile, during the spring of 1979. The disease affected almost all red apple cultivars, primarily Red King Oregon (Oregon Spur), but none of the green apple cultivar Granny Smith trees interplanted as pollinators were infected. Its occurrence in other orchards in the Central Valley since that time has been sporadic, affecting a limited number of trees.

Isolations made from the margins of surface-sterilized cankers consistently yielded one species of imperfect fungus that was identified as the *Dothiorella* stage of *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. et de Not. (syn. *B. ribis* (Tode ex Fr.) Gross. & Dug.). This fungus has previously been associated with canker diseases of the trunk on apples, peaches, almonds, and many other woody plants (1,3,5,6,10,11,14,15). It is also a major preharvest and postharvest problem of apples where warm and wet weather conditions are prevalent during the summer (4,6,12,15). Nevertheless, this fungus has not been associated previously with canker or fruit rot diseases of apples in Chile (9). This study is concerned with identification of the causal agent of the new canker of the trunk of apple trees observed in Chile and reports results obtained on evaluation of apple susceptibility to *B. dothidea*. A preliminary report has been published (8).

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MATERIALS AND METHODS

Isolations. Isolations were performed on 1.5% (w/v) potato-dextrose agar acidified with 0.5 ml/L of normal lactic acid (APDA). Cankers were surface-disinfested with 90% ethanol before removing the outer layer of bark and cutting small fragments (0.5–1 cm long) of diseased tissue from the margins of the lesions. Fragments of tissue were seeded on APDA plates and incubated at 23 C for 5–6 days. To purify the isolations, subcultures from hyphal tips were performed on APDA plates. To enhance production of sporulating pycnidia, agar plates were incubated under fluorescent light for 15 days at room temperature.

Pathogenic tests on apple, pear, and nectarine fruits. Pathogenicity of four isolates of *B. dothidea* obtained from cankers of apple trees was determined initially on cultivars Richared Delicious, Starking Delicious, and Granny Smith; pear cultivars Winter Nelis and Bartlett; and nectarine cultivar Le Grand. The inoculum consisted of disks of mycelium 0.2–0.5 cm in diameter taken from actively growing colonies on APDA. Apples were also inoculated with a conidial suspension prepared from pycnidia developed in APDA after 15–20 days of incubation at room temperature under fluorescent light. The inoculum was aseptically deposited in a hole made on surface-disinfested fruits with a sterile cork-borer 0.5 cm in diameter. Three fruits were inoculated with mycelial disks and four with a conidial suspension per each isolate. Three other fruits, wounded but not inoculated, served as controls. Fruits were incubated in a moist chamber at 25 C. These experiments were conducted three times.

Apple tree inoculations. Each of four isolates of *B. dothidea* were tested for pathogenicity on 1-yr-old Red King Oregon (Oregon Spur) apple trees. Trees were inoculated with disks of mycelium removed from edges of actively growing 5-day-old colonies on APDA. The mycelium was introduced between the inner and the outer layer of bark after making a tangential cut through the bark on the trunk, starting just above a vegetative bud. After inoculation, a polyethylene ribbon was placed around the inoculation sites. Three trees were inoculated per isolate and three wounded but uninoculated trees served as controls. All trees were maintained under field conditions for about 2 mo. The fungus was reisolated from the margins of the cankered lesions on APDA.

Apple tree susceptibility. Apple cultivars Golden Spur (Golden Delicious Spur), Granny Smith, Granny Smith Spur, Red Spur, Red King Oregon, and Starkrimson Delicious were evaluated under field conditions for susceptibility to *B. dothidea*. One-year-old trees were selected for uniformity in size from a commercial nursery and planted in an isolated experimental plot during the winter (August). Field plots consisted of two rows per cultivar, each with 10 trees planted 20 cm apart, with row spacing of 50 cm. Trees received 42 g/tree of potassium nitrate, were sprayed with sulfur for powdery mildew control, and were watered as needed.

Three isolates were used: Chilean isolates 1 and 3 and isolate 483 obtained from T. B. Sutton, North Carolina State University, Raleigh. Chilean isolates 1 and 3 were chosen based on pathogenicity results obtained previously.

The inoculum consisted of an aqueous maceration prepared from actively growing colonies on APDA. Six trees (20 November) per cultivar per each isolate were inoculated on the upper (new wood), middle, and lower parts of the trunk. Aliquots (1–2 drops) of inoculum suspension were aseptically delivered between the outer and inner layer of bark after making a tangential section with a sterile razor blade. Before inoculation, trunks were surface-disinfested with 90% ethanol. Inoculation sites were wrapped with a polyethylene ribbon. Four wounded trees per cultivar were left as controls. The lengths of the cankers were measured 30 and 67 days after inoculation. Results were analyzed statistically for

analysis of variance for a completely randomized design with a 6 × 3 × 3 factorial arrangement of treatments, respectively, for cultivar, isolate, and inoculation position. Six replicates were considered. Mean values were separated following Duncan's multiple range test.

RESULTS

Symptoms and occurrence. This disease was characterized by the development of extensive cankers, elliptical in shape, along the hardened wood (Fig. 1A) that often completely girdled the trunk of 1- to 2-yr-old trees within a few weeks. Because of canker development, the trunk above the canker eventually died. Cankers extended from the bark into the cambium and even into the sapwood. Affected tissue developed dark brown discoloration and sharp margins between healthy and diseased tissue. The surface of the cankered area was sunken and flattened. The outer bark developed an orange to purplish papery appearance and often sloughed off, exposing pycnidia (Fig. 1B). Pycnidia were almost always present on diseased bark, giving a rough appearance to the canker surface (Fig. 1A). Under some conditions, white cirrhi exuded from pycnidia immersed in the outer layer of bark (Fig. 1B). Gum exudation was not observed on affected apple trees.

Isolations. Isolations from cankers taken from apple trees in Curico consistently yielded the *Dothiorella* stage of *B. dothidea*. Identification was based on the presence of free or stromatic black pycnidia (Fig. 1A) with ellipsoid or fusoid, hyaline, and nonseptate conidia 19.5–28 μm long and 6–7.5 μm wide (Fig. 1C,D). Conidia were borne on short conidiophores (Fig. 1C). Conidia from pycnidia produced in cultures, in cankers, and on fruits were similar in size and shape. There were no differences in morphology between the Chilean and the reference isolates of *B. dothidea*.

Pathogenicity tests. All *B. dothidea* isolates were pathogenic and were reisolated from lesions produced on inoculated fruits of apple, pear, and nectarine. There were no differences in pathogenic behavior among the Chilean isolates and all produced symptoms similar to those obtained with reference isolate 483. Chilean isolate 2 was the most virulent, producing significantly ($P = 0.05$) larger lesions when apples were inoculated with either mycelium or conidia (Tables 1 and 2). Fruit symptoms consisted of necrotic lesions with definitive margins, dark brown on Granny Smith apples and light brown on red apples. Black pycnidia appeared after 10 or 15 days of incubation and matured as the fruit shriveled, dried out, and became mummified. Eventually, white tendrillike cirrhi appeared on mummified fruits.

Symptoms similar to those observed after natural infections were obtained on artificially inoculated Red King Oregon apple trees. Symptoms consisted primarily of cankers along the trunk that almost completely girdled the trunk. Flattening of the trunk, bark discoloration, and abundant pycnidia on affected tissue were also observed 45–60 days after inoculation.

Apple tree susceptibility. Rapid growth of canker lesions occurred during the first 30 days of incubation and some enlargement occurred during the second month after inoculation (Fig. 2).

Analysis of variance showed that susceptibility of apple trees to *B. dothidea* was highly dependent on cultivar and was also affected by the *B. dothidea* isolate used and varied according to the inoculation position within the tree. Highly significant effects were found for these major factors. Interactions for cultivar × isolate, cultivar × inoculation site, isolate × inoculation site, and cultivar × isolate × inoculation site were also highly significant ($P = 0.01$).

In general, the largest cankers were produced when trees were inoculated at the lower part of the trunk. Significant differences ($P = 0.05$) in mean canker length among the three inoculation positions were observed with isolate 3 on Red King Oregon, Red Spur, and Starkrimson but not on Granny Smith, Granny Smith Spur, or Golden Spur. There were no significant differences obtained with isolate 483, and differences among inoculation positions were significant ($P = 0.05$) for isolate 1 only between the upper and lower inoculations on Red King Oregon and Red Spur. Regardless of inoculation position, isolate 3 was the most virulent, producing the largest canker lesions in all six apple cultivars (Table 3) (Fig. 2). The mean canker length obtained with isolate 3, however, was significantly different ($P = 0.05$) from lesions produced by isolates 1 or 483 only when inoculations were performed at the lower part of Red King Oregon and Starkrimson apple

Table 1. Pathogenicity of four isolates of *Botryosphaeria dothidea* from Chile and one isolate (483) from North Carolina to fruit of three apple cultivars

	Diameter of decay (cm)[y]		
Isolate no.	Starking Delicious	Granny Smith	Richared Delicious
1	2.5 b[z]	3.4 b	3.1 abcd
2	7.0 a	7.0 a	6.0 a
3	3.7 b	3.0 b	1.1 d
4	5.4 ab	5.7 ab	3.4 abc
483	3.9 b	4.2 ab	5.5 ab
Checks	0.0	0.0	0.0

[y] Fruits were inoculated with 0.5-cm-diameter mycelial plugs. Results after 11 days of incubation at about 20–22 C.

[z] Numbers followed by the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

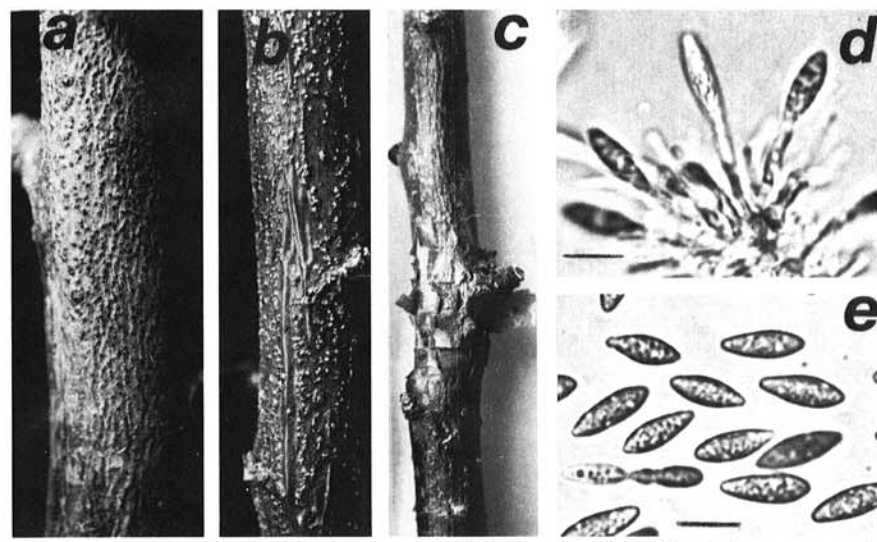

Fig. 1. *Botryosphaeria dothidea* from apples. **(A)** Abundant pycnidia, single or stromatic, extruding through the bark; **(B)** pycnidia on 1-yr-old apple stem with white tendrillike cirrhi. **(C)** Canker lesion on artificially inoculated Red King Oregon apple stem. **(D)** Conidiophores and conidia. **(E)** Ellipsoid hyaline and unicellular conidia. Scale bars = 15 μm.

Table 2. Severity of infection on apple fruits 12 days after inoculation with conidia from two isolates of *Botryosphaeria dothidea* from Chile and one isolate (483) from North Carolina

		Diameter of decay (cm)		
Isolate no.	Inoculum concentration	Starking Delicious	Granny Smith	Richared Delicious
2	1.0×10^6	4.3 a[z]	4.2 a	3.1 a
3	2.5×10^6	1.4 b	2.5 b	0.9 b
483	1.5×10^6	1.5 b	2.9 ab	0.9 b

[z] Numbers followed by the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

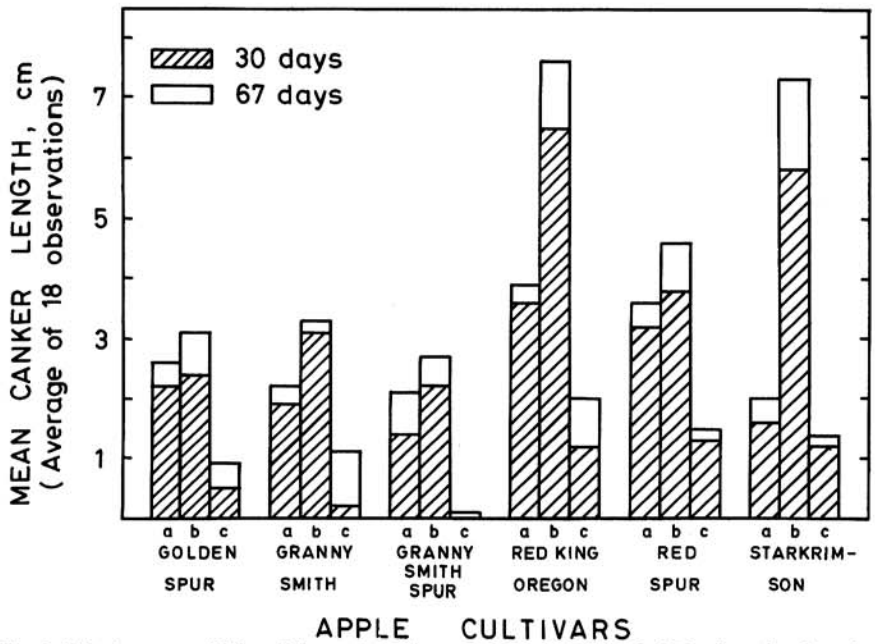

Fig. 2. Relative susceptibility of six apple cultivars to *Botryosphaeria dothidea* inoculated on the trunk with isolates 1 (a), 3 (b), and reference isolate 483 (c). Mean values represent the average of 18 observations. Three inoculations per tree, each inoculated on the upper, middle, and lower part of the trunk, replicated six times.

Table 3. Susceptibility to *Botryosphaeria* canker of six apple cultivars evaluated under field conditions

	Mean canker length (cm)[w]								
	Isolate 1			Isolate 3			Isolate 483		
Apple cultivar	U[x]	M	L	U	M	L	U	M	L
Golden Spur	1.8 a[y]	3.1 a	3.0 bc	2.3 a	3.0 bc	3.9 d	0.9 a	0.8 a	1.0 a
Granny Smith	1.9 a	2.0 a	2.6 c	2.9 a	3.4 c	3.4 d	1.5 a	1.0 a	1.1 a
Granny Smith Spur	2.0 a	2.2 a	2.2 c	2.7 a	2.7 c	2.8 d	0.1 a	0.1 a	0.0 a
Red King Oregon[z]	1.8 a	4.0 a	5.9 a	3.2 a	5.9 ab	13.7 a	1.5 a	1.9 a	2.0 a
Red Spur[z]	2.1 a	3.8 a	5.1 ab	2.8 a	4.6 bc	6.4 c	1.2 a	1.9 a	1.4 a
Starkrimson[z]	1.8 a	1.8 a	2.5 c	3.0 a	7.5 a	11.3 b	0.9 a	1.5 a	1.7 a

[w] Mean of six observations made 67 days after inoculation.
[x] Inoculations were performed on the upper (U), middle (M), or lower (L) part of 1-yr-old apple trees.
[y] Means followed by the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Red mutants of Delicious.

trees. Differences among mean values for canker lesions on the upper positions of the same cultivars were not significantly different and variable results were obtained with inoculations performed at the middle position. Small differences among isolates were obtained on Granny Smith, Granny Smith Spur, or Golden Spur and none were statistically significant at $P = 0.05$.

There were significant differences among cultivars in their reaction to *B. dothidea* isolates 1 and 3 but not to reference isolate 483 (Table 3). For instance, if we only considered the results obtained when trees were inoculated at the lower part of the trunk with isolate 3, some major differences in susceptibility among all six apple cultivars were observed. Mean values for canker length indicated that Red King Oregon was the most susceptible cultivar, followed by Starkrimson and Red Spur (Table 3). Granny Smith, Granny Smith Spur, and Golden Spur appeared to be the least susceptible cultivars. Similar results were obtained with isolate 1, except mean canker lengths were considerably smaller and Starkrimson developed rather small cankers. Mean value differences for the latter were not significant at $P = 0.05$ (Table 3).

DISCUSSION

This is the first confirmed report of *B. dothidea* infecting apples in Chile. Symptoms observed under field conditions and on artificially inoculated plants were similar to those reported on apples elsewhere (1,2,12,14) except the fruit rot phase was not found. Nevertheless, the four isolates of *B. dothidea* from Chile infected fruits of apple and nectarine. These isolates did not differ in their rotting abilities from reference isolate 483. We believe that fruit rot caused by *B. dothidea* has not been observed because of unfavorable weather conditions rather than because of the pathogenic characteristics of the Chilean isolates. Rainfall, which is essential for ascospore and conidia discharge and conidia dispersal (7,13), are very infrequent during summer months (December through March) in our apple-producing areas.

Our results indicated that red fruit cultivars, all mutant strains of Delicious, are the most susceptible to *B. dothidea*, with Red King Oregon being the most sensitive (Fig. 2). Severity of the disease depended on the isolate of *B. dothidea* used. Chilean isolate 3, originally recovered from tree cankers of Red King Oregon apples, was the most virulent, causing the largest canker lesions in all six cultivars. In contrast, reference isolate 483 was the least virulent (Table 3) (Fig. 2). As suggested by other workers (4,6,7), these results indicate that strains of *B. dothidea* may exist in nature that significantly differ in virulence. We recognize that further research is needed to clarify this point, however.

The mean canker lengths for the 7-wk-period on Red King Oregon inoculated with isolate 3 were 3.25, 5.90, and 13.70 cm for inoculations performed at the tip, middle, or lower part of the trunk, respectively (Table 3). These differences may denote the ability of this isolate to colonize hardened wood tissue. Therefore, future evaluations of apple cultivars for susceptibility to *B. dothidea* should only consider the lower part of the trunk because these inoculations were best.

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