Relationship of Botryosphaeria dothidea and Hendersonula toruloidea to a Canker Disease of Almond

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ABSTRACT

An unusual canker disease of almond (*Prunus amygdalus*) caused by *Botryosphaeria dothidea* is described. Bandlike or irregular cankers are formed on the trunk or scaffold branches of vigorous young trees, occasionally causing death of the parts distal to the point of infection. A second fungus (*Hendersonula toruloidea*) was found in many of the cankers, but in nature it appears to be mainly, if not entirely, a secondary invader. Both fungi, however, were able to induce canker formation when mycelial inoculum was placed in cortical wounds on the cambium, or on xylem exposed by pruning. Natural infection by *B. dothidea* appeared to be through cortical growth cracks. The cankers induced by both organisms were largely annual rather than perennial, and there was no evidence of a synergistic relationship between these two fungi in the formation of cankers. The mycelium of both organisms was found principally in the lumen of cells in both xylem and phloem, and it passed from cell-to-cell mostly through pits. Since the sexual stage of *B. dothidea* was not found, the identification of the almond isolate as *B. dothidea* was based on asexual stage (*Dothiorella*) morphology, serology, and pathology. The almond cultivar Nonpareil was more susceptible to canker than either Ne Plus Ultra or Mission. Canker excision, with or without a wound protectant, was of no value in disease control.

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Additional key words: pathogenicity, pathological anatomy.

Botryosphaeria ribis Gross. and Dugg. has been reported to cause branch and trunk cankers on a wide variety of woody plants (17, 18, 27). In 1954, von Arx and Müller (1) reclassified the amerosporous Pyrenomycetes and reduced *B. ribis* to synonymy with *B. dothidea* (Moug. ex Fr.) Ces. and de Not. Smith (19), working mostly with isolates of this fungus from avocado, citrus, and walnut, showed by stem inoculations that the fungus is pathogenic to 50 plant species representing 34 genera and 20 families. Species of *Prunus* found to be susceptible included *P. avium* L. (sweet cherry), *P. domestica* L. (plum), and *P. amygdalus* Batsch (almond). In Florida, *B. ribis* is reported to cause a branch canker and fruit rot of peach (21).

The imperfect fungus *Hendersonula toruloidea* Nattrass was originally found in Egypt producing dieback symptoms on plum, apricot, and apple trees (11). Wilson (25, 26) has shown it to be the cause of a serious branch wilt of walnut in California , and also has demonstrated its pathogenicity to peach and apricot. Other work has shown the ability of the fungus to attack citrus (4) and fig (14).

In 1959, an unusual canker disease of the trunk and scaffold branches of almond (*Prunus amygdalus* Batsch 'Nonpareil') trees appeared in California's Tehama and Stanislaus counties. In 1960, the disease again was observed in the same counties, and also was found in San Joaquin County. In 1961, it was found in a single orchard in Merced County, and in 1963 in a Yolo County orchard. Its occurrence in California since that time has been sporadic.

Isolations from the cankers commonly yielded two species of imperfect fungi; one has been identified as *Hendersonula toruloidea* and the second appears identical to the *Dothiorella* stage of *B. dothidea*. To the best of our knowledge, neither fungus previously has been associated with a naturally occurring canker disease of almond. The present study is concerned with the relationship of these fungi to this canker disease. An abstract covering a portion of this research has already been published (6).

MATERIALS AND METHODS.—The occurrence and development of this disease has been studied intermittently in one or more orchards in Tehama,

Stanislaus, Merced, Yolo, and San Joaquin counties since 1959. Isolates from diseased trees were used in inoculation studies and were compared with isolates of *B. dothidea* obtained from C. N. Clayton, Department of Plant Pathology, North Carolina State College, Raleigh, and with a culture of *H. toruloidea* supplied by E. E. Wilson of our own department.

After surface disinfestation of the cankers with 5% sodium hypochlorite and removal of the outer bark, small blocks of diseased phloem or xylem were aseptically transferred to 2% potato-dextrose agar (PDA) plates. All isolates were purified by the hyphal-tip method prior to their experimental use.

The pathogenicity of the isolates was determined by inoculating branches of 3- to 5-year-old almond trees with disks of mycelium-containing PDA, 5 mm in diameter, taken from the margin of actively growing colonies. Following introduction of the inoculum, plastic film was placed over the inoculation sites and tightly secured with masking tape. Control inoculations were made in a similar manner, but with sterile PDA. After various intervals, measurements were made of the length and width of the cankers.

Different types of inoculations were made and the inoculum was placed as follows: (i) at the center of the surface (xylem) freshly exposed in the pruning of almond branches; (ii) on the cambium following removal of the bark by means of a 7-mm diameter, notched cork borer (10); (iii) on the inner bark (phloem) after making a tangential cut through the outer bark; (iv) on the uninjured, external surface of smooth bark; (v) on natural growth cracks in the bark of scaffold branches; (vi) on the bark of scaffold branches directly over a vertical slit (2 cm long) extending through the bark to the cambium (simulated growth crack).

Histological study of affected tissues was accomplished by following standard fixing and embedding (paraffin) techniques, and by sectioning 6-10 μm thick. Bark and pycnidial sections were stained with 1.0% safranin in 50% ethanol and with 0.2% fast green in 95% ethanol (3); time of staining was modified for best results with each tissue type. For xylem sections the Pianese IIIb staining procedure of Wilcox (24) was used.

The effect of temperature on growth of the almond isolates was determined by transferring disks of mycelium-containing PDA, 5 mm in diameter, from the margin of actively growing colonies, to freshly poured PDA plates. The plates were incubated at a range of temperatures controlled to $\pm$ 1.0 C, and colony diameters were measured at suitable intervals.

Since only the asexual stage was observed in our isolates of *Dothiorella,* its possible relationship to *B. dothidea* was determined by antigenic comparisons with a known isolate of this fungus from apple. Our *Dothiorella* isolate 12 was used in these tests. Antigens of the two fungi were prepared from cultures grown in potato-dextrose broth at 22-24 C on a rotary shaker. The mycelia were harvested, washed with distilled water, and then extracted with 0.5 N NaCl, using approximately 10 ml of salt solution per cm^3 of wet mycelium. Antigen extractions were made by grinding the mycelia with acid-washed sand in a mortar at 2-4 C. The extracts were then clarified by centrifugation and dialyzed twice against 10 volumes of 0.14 N NaCl at 2 C. The antigens were freshly prepared every 2 weeks and stored at 2 C. Antisera were prepared by immunizing New Zealand white rabbits by intravenous injections of the antigens and also by supplementary intramuscular injections of antigen-adjuvant emulsions. The rabbits were bled and the resulting antisera frozen until used.

The agar-gel diffusion method of Ouchterlony (13) was used to determine antigenic similarities, and antibody titers of the antisera were obtained by microprecipitin tests (2). Protein concentrations of the preparations of test antigens were determined by the method of Lowry (9), and both preparations were adjusted to approximately the same concentration (120 μg protein/ml). Reciprocal absorption of antiserum was made by mixing 0.5 ml preparations of heterologous mycelial antigens with 1.0 ml of antiserum diluted to 2.0 ml with 0.14 N NaCl. Suitable checks were employed, using normal saline in place of the preparations of antigens or antisera. The mixture was incubated 4 h at 36 C and shaken at 30-minute intervals. It was then incubated for 18 hours at 2 C. Absorbed antisera were clarified by centrifugation and stored at 2 C.

RESULTS.—*Occurence and symptoms.*—This disease is largely restricted to vigorous Nonpareil almond trees 4-6 years of age. In an orchard in which tree vigor varied considerably, the disease was entirely restricted to the larger, more vigorous trees. In one instance (Stanislaus County) the disease was found in the Davey cultivar interplanted among infected Nonpareil trees, and in another case (Merced County) it was found in a single tree of the cultivar Drake. In all other observed outbreaks, the disease has been confined to Nonpareil. It has been found in one or more orchards in five important almond-producing counties in the Sacramento and San Joaquin valleys.

The disease is characterized by narrow, bandlike or irregular cankers which more-or-less girdle the trunk or scaffold branches (Fig. 1). These cankers differ from cankers of almond resulting from other causes in that their long dimension usually is transverse to the long axis of the branch or trunk. The cankers appear to have their origin in growth cracks which tend to occur in bands around the trunk and scaffold branches. They first become evident during summer and usually are accompanied by copious exudation of gum (Fig. 1-A, C). Summer canker formation has been reported in other hosts for both *B. dothidea* (8) and *H. toruloidea* (20). In older cankers of almond the bark and cambium are destroyed and, as a result of desiccation and death of the cambium, the canker usually becomes noticeably sunken. Under some conditions, minute, white spore tendrils exude from pycnidia immersed in the outer bark and are evident on the canker surface. The invaded bark turns brown and the underlying sapwood also is discolored. The latter discoloration may extend longitudinally several centimeters beyond the margin of the bark canker. In some instances, especially in very narrow cankers, the cambium is not destroyed and newly formed phloem tends to lift off the outer necrotic tissue (Fig. 1-D). Where girdling to the cambium is complete the portion of the tree above the canker eventually dies. Although complete girdling of the trunk is uncommon, one-half to two-thirds

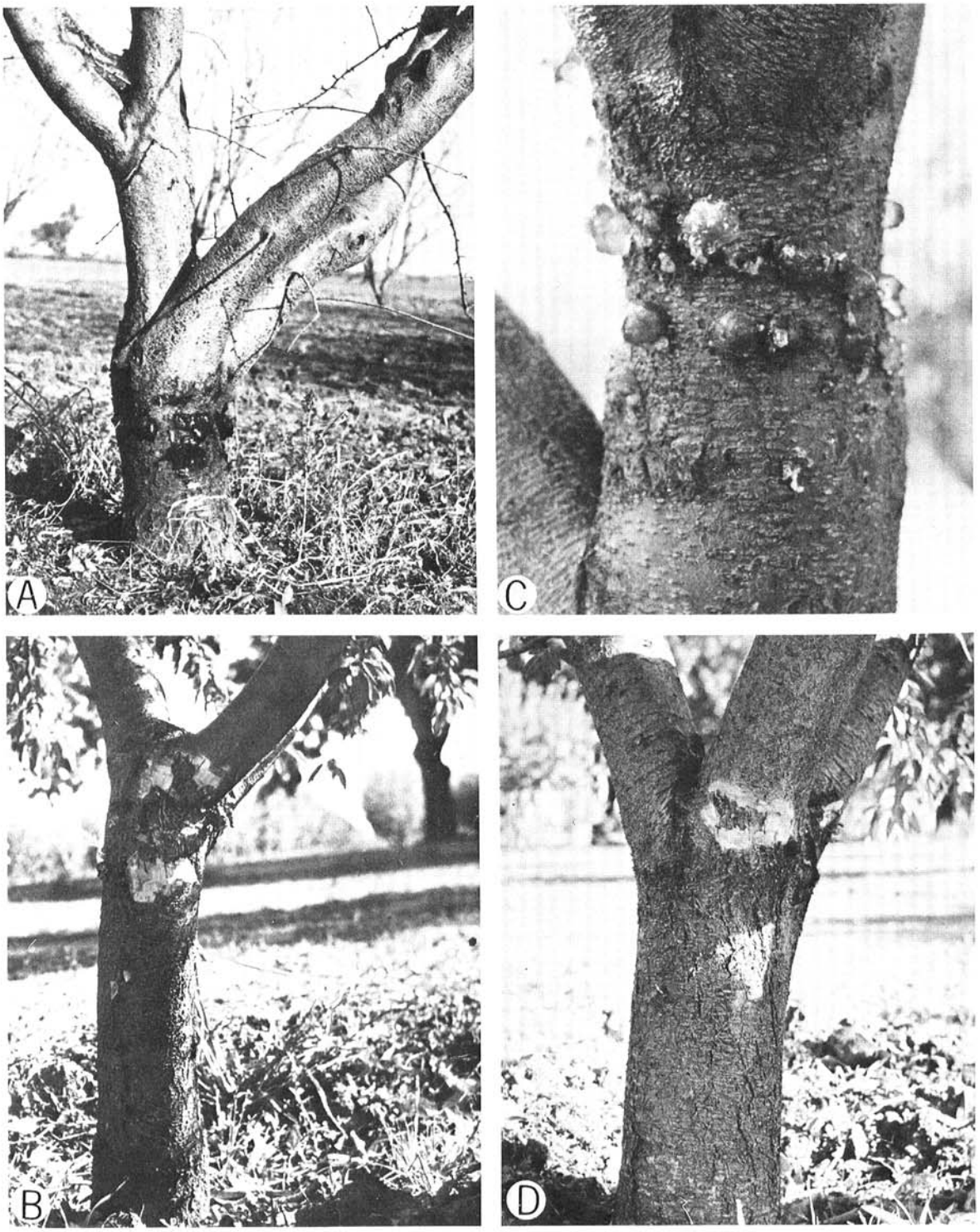

Fig. 1-(A to D). Symptoms of cankers caused by *B. dothidea* in almond trees (cultivar Nonpareil). **A)** A typical large, irregular, gummy canker on the trunk of a vigorous 6-year-old tree. **B)** A similar canker with the outer bark removed to show the extent of cortical necrosis. Dark growth cracks are evident in the cortex at the upper and lower extremities of the canker. **C)** A narrow band-type canker on the scaffold branch of a young tree. **D)** A similar canker with the outer bark removed. The cambium is not always destroyed in this type of canker.

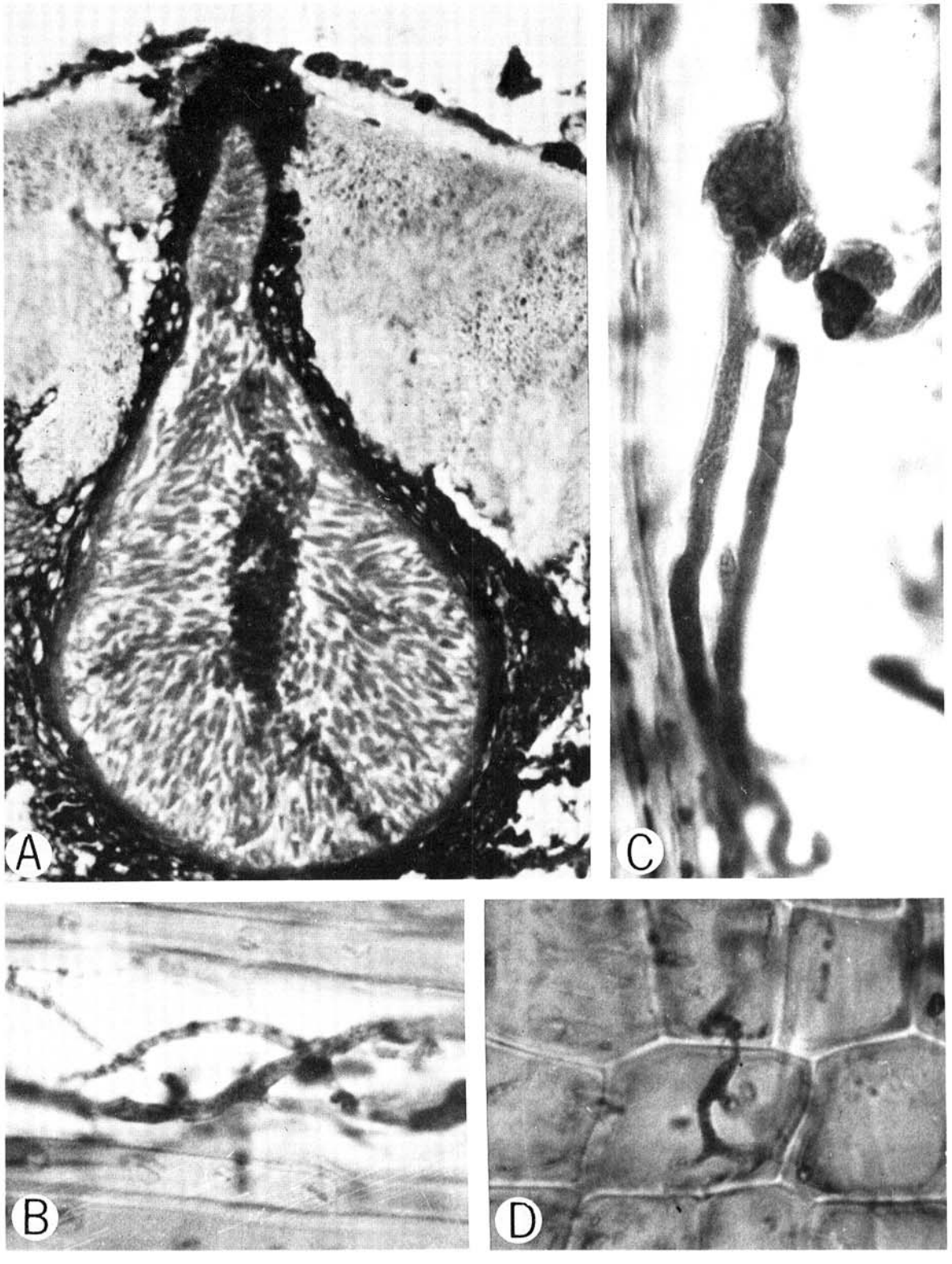

Fig. 2-(A to D). Histopathology of an almond isolate of *B. dothidea* in almond cultivar Nonpareil. **A)** Mature, stromatic pycnidium immersed in the outer bark (× 212). **B and C)** Mycelium in xylem vessels; hyphal knots (C) were occasionally seen in this tissue (B × 628, C, × 1,256). **D)** Hypha in ray parenchyma extending from one cell to another through a pit (× 628).

TABLE 1. Relationship of fungus species and time of inoculation to canker development in Nonpareil almond

Date		Canker size (mm)[a]					
		Botryosphaeria dothidea		*Hendersonula toruloidea*		*Botryosphaeria* + *Hendersonula*	
Inoculation	Examination	length	width	length	width	length	width
3 July 1961	4 Jan 1962	100.4	16.9	82.8	23.0	107.4	21.6
4 Oct 1961	4 Jan 1962	97.0	25.3	31.5	19.8	64.9	22.3
3 Jan 1962	29 Oct 1962	33.6	23.8	0.0	0.0	32.9	23.1
14 Mar 1962	29 Oct 1962	141.5	41.0	0.0	0.0	75.1	25.3

[a]Mean length and width of eight cankers. The LSD ($P=0.05$) for canker length is 26.2. Check inoculations did not produce cankers.

of the circumference often is destroyed, and scaffold branches are frequently killed. The cankers are active during the growing season, but most of them apparently do not reactivate during succeeding years. This fact, together with the infrequent occurrence of the disease, relegates it to a position of relatively minor economic importance.

Isolations.—Isolations in October, 1960, from cankers on the trunk and scaffold branches of Nonpareil trees in Stanislaus County commonly yielded two species of fungi subsequently classified in the genera *Dothiorella* (*Botryosphaeria*)and *Hendersonula*. Preliminary inoculations indicated that both organisms were able to cause rather similar cankers in Nonpareil branches. These results indicated the need for more extensive isolations to provide information on the consistency of association of these two fungi with the cankers.

Isolations from cankers collected from several San Joaquin Valley (San Joaquin and Stanislaus counties) orchards in December 1960; March and June, 1961; April, 1962; and August, 1963, yielded pathogenic isolates of *Dothiorella,* but *Hendersonula* was not recovered. In August, 1961, 47% of the cankers from the same orchards yielded *Dothiorella,* 26.5% *Hendersonula,* and 26.5% both fungi. In February, 1962, over 500 isolations were made from 10 old, apparently inactive, cankers selected from two of the above orchards. The isolations were made from various areas of the necrotic bark or wood and in some instances beyond the necrotic regions. Five of the cankers failed to yield either *Dothiorella* or *Hendersonula* (or any other recognized almond pathogen), four yielded only *Dothiorella* and one yielded both organisms. In one instance, *Dothiorella* was recovered from discolored xylem 165 mm beyond the apical margin of the bark canker. The results obtained from this sampling suggest that one or both pathogens may die in old cankers. Again in April, 1962, and July, 1963, 400 isolations were made from the margins of apparently active cankers obtained from the same orchards. The only pathogen isolated was *Dothiorella*. It was more readily recovered from discolored xylem than from affected bark.

In October, 1963, an outbreak of canker occurred in a young Nonpareil orchard in Yolo County (Sacramento Valley). From three small, apparently incipient cankers and from seven larger, more-advanced cankers *Dothiorella* was readily isolated. *Hendersonula* was not recovered in a single instance.

The preceding results indicate that *Dothiorella* was much more frequently associated with this canker condition than *Hendersonula*. Furthermore, *Dothiorella* was the only fungus found at the margins of apparently active cankers, and it was the only pathogen encountered in two of the districts where the disease was found. It appeared, therefore, that *Dothiorella* was largely responsible for this canker condition, and that *H. toruloidea* was a common secondary invader.

Identity and characteristics of the primary pathogen.—Various tests were conducted to ascertain if the primary pathogen, *Dothiorella* sp., is the imperfect stage of *B. dothidea* as represented by the isolates of this fungus from North Carolina. In one of these tests, segments of Nonpareil almond branches were placed in large test tubes containing a small amount of water, plugged with cotton and autoclaved at 1.0 atmosphere (15 lb) pressure for 30 minutes. Some of these sterilized branches were then inoculated with pathogenic *Dothiorella* isolates from almond, and others with isolates of *B. dothidea*. After incubation for 4 months under laboratory light and temperature conditions, neither group of isolates had produced fruiting bodies. The tubes were then sealed (cigarette paper) to exclude mites and moved to an outdoor shelter where they were subjected to the normal light and temperature conditions of winter. After 1 month both the almond isolates and the known cultures of *B. dothidea* had produced abundant pycnidia. Later it was observed that both groups of isolates sporulated within 2 weeks when grown on PDA and held in a shaded outdoor location during summer. They also were found to sporulate within 2 weeks when grown on PDA plates at 28 ± 1 C with 12-hour day of

TABLE 2. Effect of type of inoculation of Nonpareil almond branches on the development of cankers caused by an isolate of *B. dothidea* from almond[a]

Type of inoculation	Infection (%)	Net canker length (mm)		
		23 Oct 1961	28 Dec 1961	3 Aug 1962
Pruning wound	87.5	67.9	119.1	118.6
Cambium	68.8	68.3	72.0	73.5
Injured bark	25.0	1.8	6.2	6.1
Uninjured bark	12.5	2.9	2.9	3.0

[a]Each entry represents the mean of 16 inoculations made 26 July 1961. The LSD ($P = 0.05$) for canker length on 3 August 1962 is 65.3.

TABLE 3. Infection of Nonpareil almond scaffold branches resulting from inoculation of growth cracks or artificial slits in the bark with an almond isolate of *B. dothidea*

Type of inoculation[a]	Percent infection	Mean canker size (mm)			
		24 October 1961		28 December 1961	
		Length[b]	Width[c]	Length[b]	Width[c]
Growth cracks	37.5	33	61	33	61
Vertical slits	50.0	203	67	215	67

[a]Eight inoculations of each type were made 28 July 1961; no infection occurred in a comparable number of controls.
[b]Canker length was measured vertically; i.e., parallel to the axis of the trunk or branch.
[c]Canker width was measured horizontally; i.e., transverse to the axis of the trunk or branch.

fluorescent light at approximately 21,520 lx (2000 ft-c). The two groups of isolates responded similarly to these various ecological conditions.

A comparison of the gross morphology of the almond *Dothiorella* isolates and isolates of *B. dothidea* was made when they were grown on PDA plates, in potato-dextrose-broth shake cultures, and on sterile almond branches. Regardless of the substrate, colony appearance and gross mycelial characteristics of the two groups of isolates appeared nearly identical.

Microscopic examination of the fruiting structures of the California and North Carolina isolates also showed that they were very similar. In pycnidia produced on almond branches, our isolates formed ellipsoid to fusoid, hyaline, nonseptate pycnidiospores with average dimensions of 21.5 μm (range 12.5-33.7) $\times$ 5.9 μm (range 3.8-7.7). *B. dothidea* grown under the same conditions produced conidia of similar color and morphology that measured 24.0 μm (range 17.8-31.7) $\times$ 5.7 μm (range 4.0-7.9). With both isolates, the aging conidia often become biseptate, with the central cell brownish and the end cells hyaline. This phenomenon also has been reported for *B. dothidea* (*ribis*) by Salerno (16). Conidia of the almond *Dothiorella* germinated within 4-5 hours on water agar at room temperature. Germination was typically unipolar, but occasionally bipolar or lateral. During germination, nonseptate spores sometimes became uni- or biseptate.

Macroconidia, microconidia, and cirrhi were formed by both the almond *Dothiorella* and *B. dothidea*. These two types of conidia were found in pycnidia on inoculated almond branches as well as in vitro. The microconidia of *Dothiorella* were ellipsoid, hyaline, and had a mean size of 5.7 $\times$ 2.0 μm. Those of *B. dothidea* averaged 5.0 μm in length, but were otherwise similar to the microconidia of *Dothiorella*. Attempts to germinate the microconidia on water agar, PDA, and nutrient agar were negative.

Both the almond *Dothiorella* and *B. dothidea* produced globose-to-flask-shaped, dark-walled, mostly nonstromatic, subepidermal pycnidia on inoculated almond branches (Fig. 2-A). The pycnidia of *Dothiorella* were 238-443 μm wide $\times$ 525-574 μm high; those of *B. dothidea* were 195-361 $\times$ 417-737 μm. During humid weather, fine white cirrhi were extruded from the pycnidia. These fruiting bodies contained a wall layer of simple, hyaline conidiophores that measured approximately 7-8 $\times$ 1-2 μm. Neither *Dothiorella* nor *B. dothidea* were observed to produce perithecia either in vitro or in vivo.

The relation of temperature to mycelial growth of the almond *Dothiorella* isolates agrees, in general, with that reported for *B. dothidea* (22, 27). The optimum temperature for growth of our isolates was 24-30 $\pm$ 0.5 C. Following a 26-day incubation period, our isolates were viable at 1.0 $\pm$ 0.5 C and 37.0 $\pm$ 0.5 C, but no growth had occurred at these temperatures. The minimum and maximum temperatures at which growth occurred were 8.5 $\pm$ 0.5 C and 31.5 $\pm$ 0.5 C, respectively.

Serological comparisons of the almond *Dothiorella* with a known isolate of *B. dothidea* strongly suggested that these isolates are identical. Agar-gel double diffusion tests, involving cross reactions between homologous and heterologous antisera and preparations of antigens, yielded patterns of precipitin bands which showed the similarity of the various antigens from the fungal isolates. Microprecipitin tests additionally support this close antigenic relationship of the fungal isolates. Finally, cross-absorption of antisera with heterologous preparations of the test antigens eliminated the formation of precipitin bands between the cross-absorbed antisera and the homologous and heterologous antigen preparations.

Pathogenicity tests were also conducted to provide further information on the relationship of our *Dothiorella* isolate to *B. dothidea*. The trunks of 2-year-old Ne Plus Ultra almond trees were inoculated at the cambium with two isolates of *B. dothidea* and one of *Dothiorella*. The trees were maintained in a greenhouse until canker symptoms had developed. Both organisms were pathogenic, and the symptoms they induced were almost identical.

Based on the above morphological, serological, and pathological studies, it is concluded that the almond *Dothiorella* is, in fact, the imperfect stage of *B. dothidea*.

TABLE 4. Relative susceptibility of three almond cultivars to an almond isolate of *B. dothidea*[a]

Cultivar	Infection (%)	Canker size (mm)	
		Length	Width
Nonpareil	100	99.4	24.1
Ne Plus Ultra	63	47.5	13.8
Mission	25	10.1	5.3

[a]Data recorded represent the means of 8 cambial inoculations made 3 July 1961, and measured 21 March 1962. The LSD (P = 0.05) for canker length is 47.3.

Pathogenicity of almond isolates of Botryosphaeria and Hendersonula to Nonpareil almond.—The relative virulence of *Botryosphaeria dothidea* (isolate 12) and *Hendersonula toruloidea* (isolate 42) to Nonpareil almond branches at various times of the year was determined. The organisms were inoculated separately and in combination by placing mycelium-containing disks of PDA on the exposed cambium of branches approximately 2-3 cm in diameter. The bark cankers were measured at various intervals until no further enlargement was observed. *B. dothidea* was highly virulent when inoculations were made in spring, summer, or fall and weakly virulent in winter (Table 1). *H. toruloidea,* however, was highly virulent only in summer and was unable to induce disease in January or March. These results are not surprising, in view of the high temperature required for growth of this fungus in vitro (25). Inoculations with combined inoculum of both fungi did not enhance canker formation. In fact, in the combination inoculations made on 4 October 1961, and 14 March 1962, *Hendersonula* interfered with the ability of *Botryosphaeria* to cause cankers. The failure of *H. toruloidea* to cause cankers from inoculations on 3 January 1962 and 14 March 1962 could not be attributed to loss of virulence, because later inoculations showed this isolate to be highly virulent. Although this fungus is pathogenic when artificially inoculated into almond trees, our evidence indicates that in nature it is largely, if not entirely, a secondary invader.

Susceptibility of different branch tissues to infection and canker development.—Pruning wounds (xylem) and exposed cambium were found to be highly susceptible to infection by *B. dothidea* (isolate 12), whereas injured or uninjured bark was highly resistant (Table 2). Cankers resulting from pruning-wound and cambial infections developed rapidly in summer and early fall, but their progress was halted in late fall or early winter. These cankers did not continue to develop the following year, which supports field observations that most cankers appear to be annual rather than perennial. Control inoculations showed no infection.

In a similar series of inoculations with *H. toruloidea* on 1 May 1962, cankers resulting from cambial infection had a mean size of 53.3 × 22.1 mm on 6 July. They showed no further enlargement that year nor by August of the following year. Cankers less than half this size resulted from inoculations into the bark or in pruning wounds, and these too showed no further increase in size after 6 July. Inoculum placed on the surface of uninjured bark and control inoculations resulted in no infection.

A moderate amount of infection was obtained when the inoculum of *B. dothidea* was placed on vertical slits (artificial) or natural growth crack zones in the bark of scaffold branches (Table 3). Vertically elongate cankers resulted from the slit inoculations whereas horizontally elongate cankers developed when the inoculum was placed on growth cracks. The growth crack areas, which often occur as narrow bands around the trunk or scaffold branches, appear to be more susceptible to infection than the normal cortex and thus promote the lateral elongation of the cankers. These artificially induced cankers were similar in shape to many of the small, naturally occurring cankers. Here again, the cankers became inactive in late fall or early winter, and no further enlargement occurred the following year.

Relative susceptibility of almond cultivars to B. dothidea.—Cambial inoculations have shown that Nonpareil is considerably more susceptible to infection and invasion than either the Ne Plus Ultra or Mission cultivars (Table 4). In nature, this disease occurs almost exclusively in Nonpareil, and the results of this test suggest that both Ne Plus Ultra and Mission have some internal resistance mechanism.

Pathological anatomy of cankers induced by almond isolates of B. dothidea and H. toruloidea.—Cankers on 1- to 2-year-old branches of Nonpareil almond, resulting from cambial inoculations on 4 October, were examined histologically on 17 November. At this time, most cankers had ceased to enlarge. The cankers caused by *H. toruloidea* were depressed and elliptical in shape with the long dimension parallel to the longitudinal axis of the branch. By contrast, the cankers induced by *B. dothidea* were more irregular in shape and somewhat less sunken. They also showed the presence of erumpent pycnidia with conidial cirrhi. No sporulation was observed on cankers formed by *H. toruloidea.* With both pathogens, the necrotic cortical tissue beneath the periderm was brown, with a light-colored, water-soaked margin, but without distinct zonation (Fig. 1-B, D). Beneath the necrotic cortex, the xylem showed a brownish discoloration that often extended longitudinally several centimeters beyond the cortical margin. With *B. dothidea,* small isolated cankers occasionally developed some distance above the canker produced at the point of inoculation. Histological observations and isolations demonstrated that these secondary cankers arose from vascular mycelium that had extended well beyond the margin of the primary cankers.

The mycelium of both pathogens ramified through the cortical tissues and sieve tubes, but did not extend beyond the visible margin of the cankers. It appeared to penetrate the cells by means of the pits (Fig. 2-D) and was largely intracellular. Hyphae were not observed in the phellogen or the phellem. In the xylem, mycelium of both pathogens was observed in vessels, tracheids, and ray parenchyma (Fig. 2-B, C). Both tyloses and gum deposits were evident in vessels of infected xylem. No evidence was obtained that cell wall materials were being decomposed.

Control.—Attempts to control this disease by means of canker excision were made in December, 1960 before it had been established that most cankers are annual rather than perennial. All of the necrotic bark tissue down to the cambium was removed but no attempt was made to remove the discolored xylem which sometimes extended beyond the canker margin. Some of the excavated areas were treated with a wound protectant containing a mixture of 8 parts glycerol, 2 parts anhydrous lanolin, and 0.3% phenylmercuric nitrate (5); others were left untreated. Nonexcised cankers, whose margins were outlined with a wax pencil, served as checks. The test was conducted in two orchards in the San Joaquin Valley, and results were assessed at intervals during the ensuing 18 months.

Some of the cankers, both those that had been excised and the controls, showed evidence of enlargement when examined in June, 1961, but by May, 1962, healthy callus

had formed at the margin of almost all of the excised and control cankers. Data taken in August, 1961, indicated that 62% of the excised canker sites no longer were active, and that 57% of the control sites also were inactive. The wound protectant had no effect on canker enlargement. The reason canker excision (bark only) failed to arrest canker development is probably due to the fact that viable mycelium of the pathogen is often present in the xylem some distance beyond the bark canker margin. Measures designed to protect the tree against infection have not been investigated.

DISCUSSION.—This new disease of almond is unusual in that the long dimension of the cankers tends to be lateral rather than longitudinal, and in that many of the cankers contain two unrelated fungi both of which are pathogenic to the host under artificial conditions. In most fungus canker diseases of woody plants the long dimension of the canker is oriented with the long axis of the branch or trunk, and cankers in nonalmond hosts caused by *B. dothidea* usually have this orientation (7, 15). Our results suggest that the more rapid lateral development of natural cankers in almond results from the increased susceptibility of the cortical tissue in growth-crack bands frequently found on vigorous young trees. When cambial inoculations were made into artificial injuries in smooth bark, the cankers elongated longitudinally. *B. dothidea* is primarily a wound-invading organism (7, 15, 17, 27), and the cortical growth cracks appeared to serve as suitable infection courts.

Although both *B. dothidea* and *H. toruloidea* were frequently present in almond cankers, and both fungi were shown to be able to produce cankers in this host, our evidence strongly indicates that *B. dothidea* is the primary pathogen and *H. toruloidea* is a common secondary invader. Both of these fungi have been associated with canker diseases in citrus (12), walnut (19, 25), and apple (11, 18), but both pathogens apparently have not been present in the same canker. Although Smith (19) showed by artificial inoculation that *B. dothidea* was pathogenic to almond, our study is the first to describe a naturally occurring canker disease of almond caused by this fungus.

Although the perfect stage of *B. dothidea* was found neither in vitro nor in vivo, there seems little doubt that the *Dothiorella* sp. we isolated is the imperfect stage of this Pyrenomycete. This conclusion is supported by morphological, serological, and pathological comparisons of our isolate with an authentic culture of *B. dothidea*. The infrequent occurrence of the sexual stage of this fungus in infected host plants and/or in culture has been reported by a number of investigators (18, 23, 27).

Both *B. dothidea* and *H. toruloidea* were able to readily infect the cambium or xylem of almond branches, but neither fungus was able to cause much infection of artificially injured cortical tissues. *H. toruloidea* appeared to be extremely temperature-sensitive, as has been reported for this fungus in walnut (20), and was able to cause appreciable infection only in summer. *B. dothidea,* on the other hand, was highly virulent in spring, summer, and fall. The mycelium of both organisms was largely in the lumen of cells in both the xylem and phloem, and grew from cell-to-cell mainly through pits. Cankers induced in almond by both pathogens appear to be principally annual rather than perennial. However, cankers in walnut induced by *H. toruloidea* (25) and cankers caused by *B. dothidea* in a number of hosts (8, 17) are reported to enlarge year after year. On the other hand, cankers induced by *B. dothidea* in linden and redbud are reported to be only annual (23).

A number of questions pertinent to the development and control of this disease remain unanswered. The source and dispersal of inoculum, environmental conditions favorable for infection, and the time of infection have not been investigated. Persian walnut trees occur in close proximity to some of the infected almonds and, since walnuts are known to be susceptible to both *B. dothidea* and *H. toruloidea* (25), it is possible that they constitute an important source of inoculum. Another aspect of the disease that remains largely untouched is its control. The present relatively low incidence and severity of the disease, however, do not appear to warrant further extensive investigation.

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