

# INOCULATIONS SHOWING THE WIDE HOST RANGE OF *BOTRYOSPHAERIA RIBIS*<sup>1</sup>

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## INTRODUCTION

The organism, *Botryosphaeria ribis*,<sup>2</sup> which causes the cane blight of currant, *Ribes rubrum*, *R. americanum*, *R. nigrum*, and *R. aureum*, has a wide distribution. It is found in parts of the United States, Canada, Hawaii, Palestine, Algeria, Tunisia, and Australia. It is omnivorous in its host relations and has been found on plants belonging to a number of different species.

## HISTORY AND HOST RANGE

The importance of *Botryosphaeria ribis* as a pathogene on currant and gooseberry, *Ribes grossularia*, was demonstrated by Grossenbacher and Duggar (7).<sup>3</sup> They described the perfect stage as *B. ribis*. The imperfect stage, which is common on most hosts, is a *Dothiorella*. Later, Shear, Stevens, and Wilcox (17) verified their work and studied critically (using over 3,000 cultures) the closely related fungi collected from 26 different hosts.

Shear, Stevens, and Wilcox (18) report 17 species of plants in the United States as hosts of *Botryosphaeria ribis*. Stevens (20) gives almost the same list, with additional species. Stevens and Shear (22) found *B. ribis* to be relatively scarce in the Hawaiian Islands. They made less than 30 collections, and list its occurrence on 14 species of plants. They grew cultures of the fungus from 15 of their collections, and proved the pathogenicity on currants of 5 of these cultures selected at random.

Saccardo (16) lists and describes 72 different species of the genus *Dothiorella* that were found on 54 different genera, and on a greater number of species.

Fawcett (1, 2) found a species of *Dothiorella* attacking limbs of the English walnut, *Juglans regia*, named the disease melaxuma, and discovered that the arroyo willow, *Salix lasiolepis* Benth., had typical cankers caused by the same fungus. This fungus, from its imperfect form, was provisionally determined as *Dothiorella gregaria* Sacc. which is now believed to be identical with the *Dothiorella* stage of *Botryosphaeria ribis* on currant and gooseberry, as described by Grossenbacher and Duggar (7). A decay of citrus fruits, especially of lemons, is listed by Fawcett (5, pp. 412-414) as caused by *B. ribis*. He reports *Dothiorella* sp. as girdling and killing large citrus branches, in Italy (3, p. 132), in Tunisia (4, p. 208), and in Algeria (4, p. 244). Reichert and Hellinger (15) record *B. ribis* as the cause of decay of

<sup>1</sup> Received for publication Mar. 16, 1934; issued September, 1934. Paper no. 293, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

<sup>2</sup> The name *Botryosphaeria ribis* is used in this paper for strains agreeing with *B. ribis chromogena* Gros. and Dug.

<sup>3</sup> Reference is made by number (italic) to Literature Cited, p. 475.

oranges, and as the cause of bark lesions on citrus in Palestine. Shear, Stevens, and Wilcox (17), in 1917, inoculated grapefruit with the currant cane-blight organism and produced a typical rot. Stevens and Jenkins (21) studied a blight on roses and a decay of the fruit of the horsechestnut and proved that it was caused by *B. ribis*. Putterill (14) describes a canker of the apple tree in South Africa that was caused by a fungus for which he proposed the name *Botryosphaeria mali*. Shear, Stevens, and Wilcox (17) believe that this fungus is not different from *B. ribis*. Fenner (6) describes a rot of apples caused by *B. ribis*. Horne (8, 9, 10, 11, 12) finds a *Dothiorella* to be one of the causes of the spoilage of mature avocado fruits. It is also believed to be the same species as *B. ribis*. Stevens (19) reports a species of *Botryosphaeria* (probably *B. ribis*) on avocado from Cuba.

The following are some of the hosts attacked by *Botryosphaeria ribis*, as reported in the literature: *Acalypha* sp. (22), *A. marginata* (20); *Aesculus hypocastanum* (20, 21); *Aleurites moluccana* (22); *Amygdalus* sp. (18), *A. persica* (20); *Baccharis* sp. (18, 20); *Berberis* sp. (20); *Cassia* sp. (20); *Citrus sinensis* (5), *C. limonia* (5); *Eucalyptus* sp. (18, 22); *Gossypium* sp. (18), *G. hirsutum* (20); *Hibiscus sabbdariffa* (22), *H. tiliaceus* (22); *Hicoria pecan* (20); *Juglans* sp. (18), *J. regia* (1, 2); *Laguncularia* sp. (18), *L. racemosa* (20); *Leucaena glauca* (22); *Liriodendron* sp. (18), *L. tulipifera* (20); *Liquidambar* sp. (18), *L. styraciflua* (20); *Magnolia* sp. (20); *Mangifera indica* (22); *Melia* sp. (18); *Pandanus odoratissimus* (22); *Persea* sp. (8, 9, 10, 11, 12, 20, p. 208); *Pipturus albidus* (22); *Poinciana* sp. (18), *P. pulcherrima* (20); *Psidium guajava* (22); *Pyrus malus* (6, 14, 17); *Quercus* spp. 18, 20; *Ribes* sp. (7, 17, 20); *Ricinus* sp. (18), *R. communis* (20); *Rosa setipoda* and other hybrids (21); *Rubus* sp. (19) raspberry; *Sambucus* sp. (18, 20); *Salix* spp. (20); *Schinus molle* (22), *S. terebinthifolius* (22); *Vitis* sp. (18); *Wikstroemia phillyreaefolia* (22).

The occurrence in California of a similar *Dothiorella* on Citrus; avocado, *Persea americana*; and walnut *Juglans* sp.; and its isolation from *Cocos plumosa* Hook. suggested cross-inoculation experiments on these hosts with strains isolated from these four hosts. The plan was later modified and made to include inoculations on species of 25 different genera.

#### MATERIAL AND METHODS

Two strains of *Dothiorella* were isolated from English walnut, *Juglans regia*. One strain was obtained from the pycniospores on a dead shoot, the other from an active melaxuma canker. Cultures of the latter usually caused larger lesions than any other strain tested. The avocado strain was furnished by W. T. Horne, and was isolated from a Fuerte avocado secured from the coastal region. The citrus culture was supplied by H. S. Fawcett, and was isolated from lemon bark. The palm strain was isolated by L. J. Klotz from *Cocos plumosa*.

*Botryosphaeria ribis* is probably of rare occurrence on any of the Palmaceae as this is the first time that the disease has been found in California. This diseased specimen of *Cocos plumosa* was growing in a city lawn which had been sprinkled with a spray that splashed the water on the trunk and gave exceptionally favorable conditions for fungus infection. The palm was of good size with a trunk at base about 1.5 feet in diameter. These natural infections consisted of

numerous black lesions that were 1 to 6 inches in diameter and 1 to 1.5 inches deep (pl. 3, A). They were for the most part arranged in a straight line that extended from near the base of the palm to a height of about 6 feet, but some other lesions were found at other points without any definite arrangement.

The method of inoculation consisted in placing a pure culture of the organism from glucose-potato agar in injuries in the bark of trees growing in the open. Uniform wounds were secured by making cylindrical holes with a cork borer, 7 mm in diameter. After the insertion of the fungus, the excised bark was replaced and the whole wrapped with nurseryman's tape. This method of inoculation is similar to that described by Klotz and Fawcett (13) and Wright (23). On small twigs a slight tangential oblique cut into the tissue was made, the inoculum placed in the incision, and the twig wrapped with tape. All the inoculations except some later ones on the Palmaceae were protected with nurseryman's tape. These other inoculations on the Palmaceae were made by placing the inoculum over the injury and moist cotton over the inoculum. Then all was wrapped with paraffin paper whose ends were fastened to the plant tissue with tape. Control experiments were run at the same time as the inoculations. They were made on the same or an adjacent twig, were wounded and protected in the same manner as the inoculations.

#### INOCULATION AND RESULTS

Inoculations with controls were made on different species of Rosaceae, Juglandaceae, Palmaceae, and on *Citrus*, *Persea* (avocado), and other genera. The results, although somewhat variable, were consistent in that nearly all the species showed some evidence of susceptibility. Some results were negative. These, before being considered as conclusive, should be confirmed by repeating the inoculations. The size of the diseased areas was judged by measuring the longest radius of blackened or discolored tissue, in millimeters, from the margin of the infection court. A radius less than 5 mm does not have much pathogenic significance. The results of inoculation on the hosts used are tabulated in tables 1 to 4 under the family names Rosaceae, Juglandaceae, and Palmaceae, the genera *Citrus* and *Persea*, and a miscellaneous group of species. The species that have not been reported in literature as hosts of *Botryosphaeria ribis* are marked in tables with an asterisk.

TABLE 1.—Results of inoculations with *Botryosphaeria ribis* on plants belonging to Rosaceae, Juglandaceae, and *Citrus*

#### ROSACEAE

Hosts inoculated <sup>1</sup>	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	Number	Number	Millimeters
*Almond ( <i>Prunus communis</i> ).....	Walnut.....	35	4	4	25-50
Do.....	Avocado.....	61	6	3	10-25
Apple ( <i>Malus sylvestris</i> ).....	Walnut.....	33	8	7	3-90
Do.....	Avocado.....	21	4	4	30-45
Do.....	Palm.....	25	3	2	15-25
Apple (fruit).....	Walnut.....	16	2	2	20-30
*Apricot ( <i>Prunus armeniaca</i> ).....	do.....	10	10	6	15-35
Do.....	Avocado.....	40	8	4	25-50

<sup>1</sup> Species that have not been reported so far as the author knows in literature as hosts of *Botryosphaeria ribis* are marked with an asterisk (\*).

TABLE 1.—Results of inoculations with *Botryosphaeria ribis* on plants belonging to Rosaceae, Juglandaceae, and Citrus—Continued

## ROSACEAE—Continued.

Hosts inoculated	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	Number	Number	Millimeters
Blackberry ( <i>Rubus</i> sp.)	Walnut	10	4	3	20-30
Blackberry (Mammoth)	Citrus	15	3	2	15-20
Blackberry ( <i>Rubus</i> sp.)	Avocado	10	5	3	10-20
Do	Palm	25	3	3	5-10
*Cherry ( <i>P. avium</i> )	Walnut	36	7	7	10-70
Do	Avocado	62	3	3	(?)
*Cherry ( <i>P. serotina</i> )	Walnut	25	2	2	40-50
* <i>Cotoneaster pannosa</i>	do	40	3	2	30-90
Do	Avocado	40	3	3	10-30
Heteromeles arbutifolia	Walnut	22	3	3	20-40
Do	Avocado	22	3	3	5-25
*Loquat ( <i>Eriobotrya japonica</i> )	Walnut	22	3	2	20-30
Do	Avocado	24	3	2	15-25
Peach ( <i>Amygdalus persica</i> )	Walnut	23	3	3	25-60
Do	Avocado	23	4	3	30-35
Do	Citrus	27	4	3	5-10
*Pear ( <i>Pyrus communis</i> )	Walnut	36	3	3	70-80
*Plum ( <i>Prunus domestica</i> )	Avocado	48	3	2	25-30
*Plum ( <i>P. salicina</i> )	Walnut	50	4	4	30-100
* <i>Pyracantha gibbsii</i>	do	20	3	3	20-40
<i>P. yunnanensis</i>	do	20	3	3	5-15
<i>Rosa</i> sp. Lady Hillingdon	do	18	3	3	15-35
<i>Rosa</i> , Maman Cochet	Avocado	18	3	3	10-20
<i>Rosa</i> sp.	Palm	25	3	3	10-15
*Quince ( <i>Cydonia oblonga</i> )	Walnut	25	7	6	20-100
Do	Avocado	25	4	3	10-25
Do	Palm	25	2	2	10
Do	Citrus	30	2	0	-----

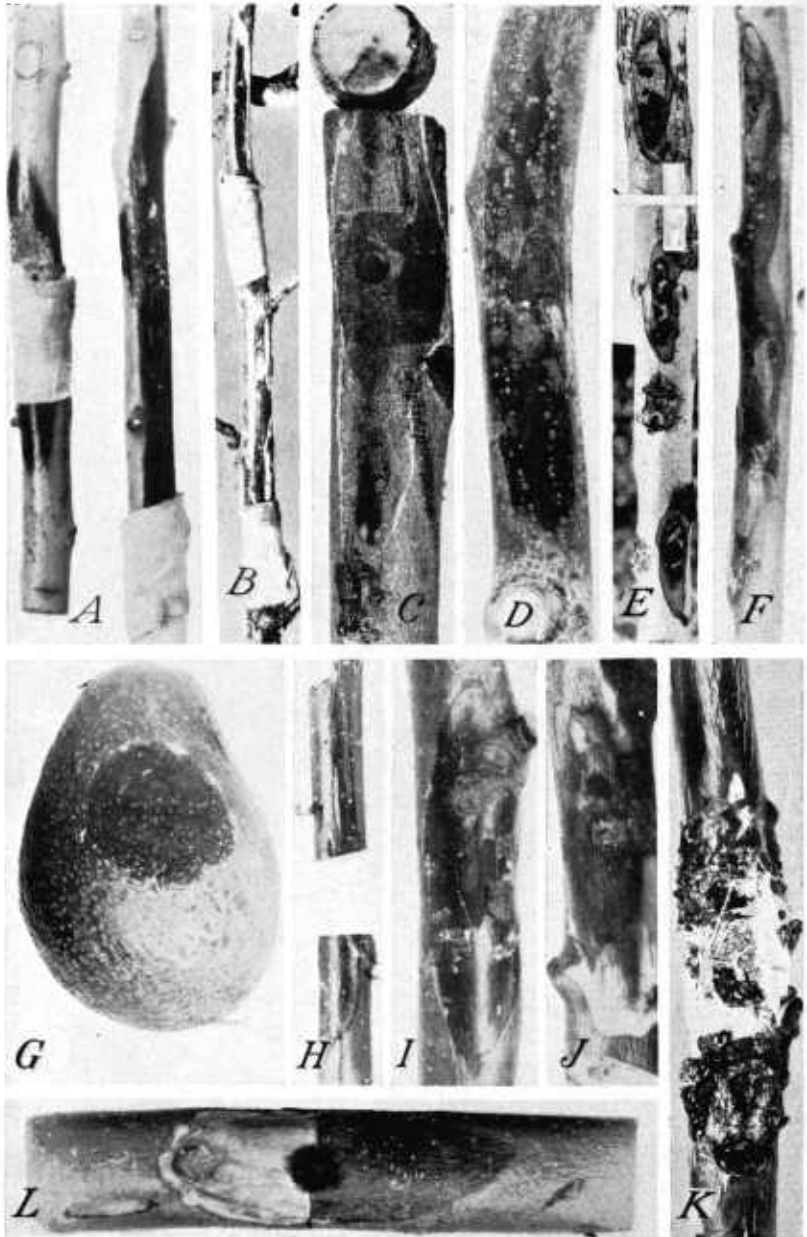
## JUGLANDACEAE

* <i>Juglans nigra</i>	Walnut	45	12	3	5-10
Do	Avocado	36	6	0	-----
Do	Citrus	33	3	2	5-5
<i>J. regia</i>	Walnut	45	4	4	30-40
Do	Avocado	45	5	5	10-25
Do	Palm	25	3	3	25-60
* <i>J. californica</i>	Walnut	45	5	4	5-20
Do	Avocado	45	2	0	-----
* <i>J. hindsii</i>	Walnut	45	4	4	5-10
Do	Avocado	45	3	2	4-5
* <i>J. major</i>	do	30	3	3	10
Do	Walnut	30	3	3	20-30
* <i>J. sieboldiana</i>	Avocado	45	3	2	5-10
<i>Carya pecan</i>	do	24	2	2	3-5
Do	Walnut	60	2	2	10-50
* <i>Pterocarya fraxinifolia</i>	Avocado	20	2	2	3-5
Do	Walnut	20	5	2	5-7

## CITRUS SHOOTS AND FRUITS

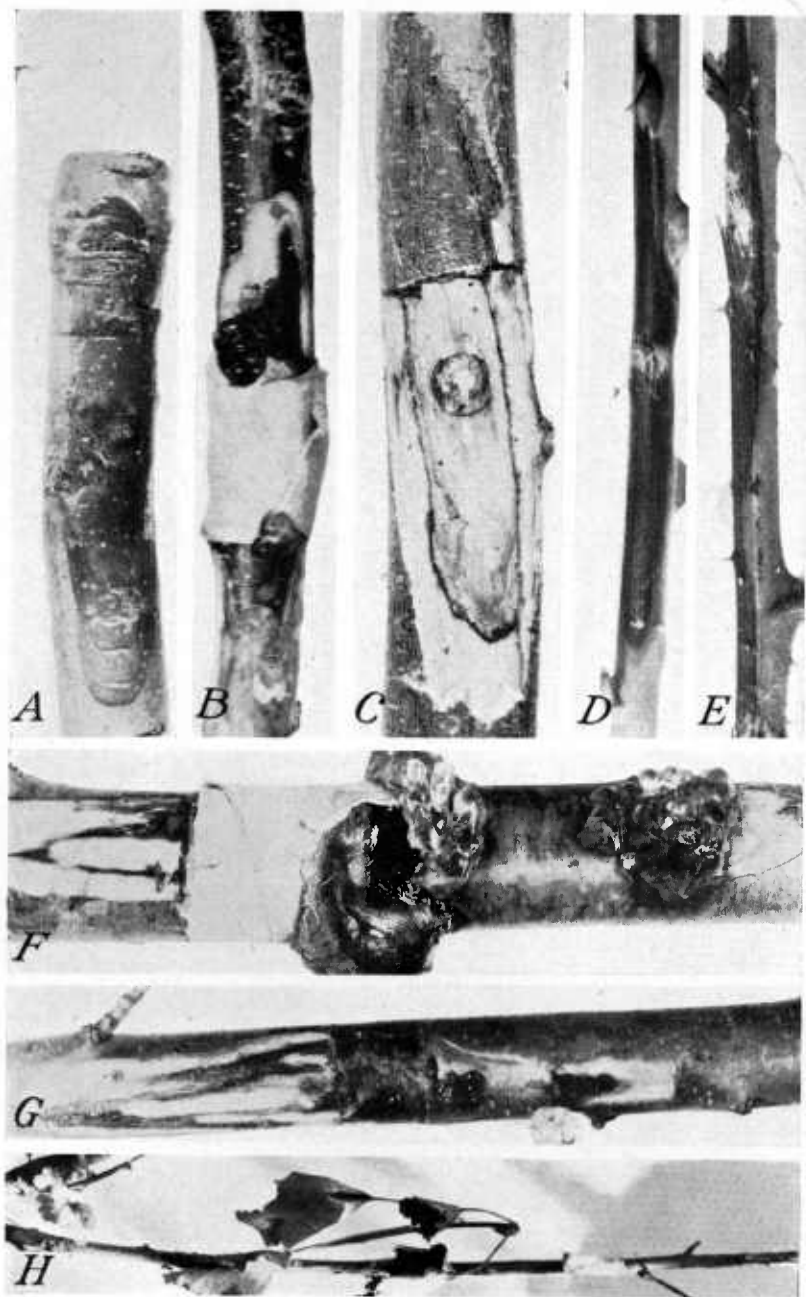
Citrus shoots:					
<i>Citrus sinensis</i> , Washington Navel	Walnut	45	4	4	5-7
<i>C. sinensis</i> , Valencia	do	13	6	6	40-70
Do	Palm	25	2	2	5
<i>C. grandis</i> , Marsh	Walnut	13	3	3	15-60
Do	Avocado	30	3	3	30-35
<i>C. limonia</i> , lemon	Walnut	26	3	3	20-60
Do	Avocado	45	4	4	10-15
<i>C. aurantium</i> , sour	Citrus	27	3	2	3-5
Do	Walnut	36	10	4	10-15
Do	Avocado	36	4	0	-----
Citrus fruits:					
<i>Citrus sinensis</i> , Washington Navel	Walnut	10	1	1	50
Do	Avocado	12	1	1	15
<i>C. sinensis</i> , Valencia	Walnut	12	1	1	70
Do	Avocado	12	1	1	50
<i>C. grandis</i> , Marsh	Walnut	12	2	2	70-140
Do	Avocado	12	2	2	25-70
<i>C. limonia</i> , lemon	Walnut	12	2	2	25-50
Do	Avocado	12	2	2	25-50

<sup>1</sup> Girdled.



ARTIFICIAL INOCULATIONS ON DIFFERENT HOSTS WITH *BOTRYOSPHAERIA RIBIS* FROM CITRUS, AVOCADO, AND WALNUT.

A, Avocado strain on stems of *Persea americana* (avocado). B, Walnut strain on *Corylus avellana* L. (filbert). C, Walnut strain on *Eucalyptus* sp. after 50 days. D, *Carya pecan* inoculated with walnut strain. E, Walnut strain on *Juglans major* after 60 days. F, Walnut strain on *Citrus grandis*, Marsh grapefruit. G, Fruit of Guatemalan avocado, *Persea americana*, inoculated with walnut strain of *Dothiorella* sp. in contact without wounds. H, Walnut strain on *Castanea* sp. after 27 days. I, Inoculation on *J. regia* with walnut strain. J, Walnut strain on *Annona cherimola*. K, Valencia orange, *C. sinensis*, inoculated with walnut strain May 5, photographed October 5, 1933. L, Walnut strain on *Olea europaea* after 50 days.



ARTIFICIAL INOCULATION ON *RIBES VULGARE* AND PLANTS OF THE ROSACEAE WITH CULTURES OF *BOTRYOSPHAERIA RIBIS* ISOLATED FROM WALNUT, AVOCADO, AND CITRUS.

- A, Walnut strain inoculated in twigs of *Pyrus malus*, photographed after 32 days. B, Walnut strain on *Prunus armeniaca* (Royal apricot) after 64 days. C, Walnut strain on *Cydonia oblonga* Mill. (quince), photographed after 51 days. D, *Rosa* sp. (Lady Hillingdon) inoculated with walnut strain, photographed after 25 days. E, Inoculation with walnut strain of *Dothiorella* sp. on Mammoth blackberry *Rubus* sp. F, Walnut strain inoculated on sweet cherry, *Prunus avium*, 60 days. G, Walnut strain on *P. serotina* Ehrh., photographed after 22 days. H, currant, *R. vulgare*, 64 days after inoculation with walnut strain of *Dothiorella* sp.

The genera of the Rosaceae (table 1) inoculated were *Cotoneaster*, *Cydonia*, *Eriobotrya*, *Heteromeles*, *Rosa*, *Rubus*, *Prunus*, *Pyrus*, and *Pyracantha*. In varieties of *Prunus domestica*, *P. avium*, *P. communis*, and *P. persica*, definite infected areas developed. A large amount of gum was often present, whether the results were positive or negative (*Citrus*, pl. 1, and *Prunus*, pl. 2). Two varieties of rose, Lady Hillingdon and Maman Cochet, were especially susceptible and developed diseased areas having a radius of 10 to 35 mm in 18 days (pl. 2, *D*). On Mammoth blackberry, positive lesions 10 to 30 mm in radius were formed (pl. 2, *E*). The apple and pear readily developed cankers 30 to 80 mm in radius in about 30 days (pl. 2, *A*). The loquat, *Eriobotrya japonica*, the quince, *Cydonia oblonga*, the California Christmasberry, *Heteromeles arbutifolia*, and firethorn, *Pyracantha gibbsii* and *P. yunnanensis*, were all readily infected. The quince (pl. 2, *C*) and loquat were especially susceptible and had infected areas 20 to 50 mm in radius in 30 days.

Of the Juglandaceae tested, the various walnuts *Juglans regia*, *J. californica*, *J. hindsii*, *J. major* (pl. 1, *E*), and *J. sieboldiana* were all susceptible and had diseased areas with a radius of 5 to 40 mm. *J. regia* had the largest canker, ranging from 30 to 40 mm in radius in 45 days (pl. 1, *D*). The diseased areas on the other species were 5 to 20 mm in radius. *J. nigra* showed negative results in 15 inoculations and small lesions (5 mm radius) in 5 inoculations. The inoculations on *Pterocarya fraxinifolia* were of small size. On *Carya pecan* the infected areas were 10 to 15 mm in radius in 24 days, increasing to 50 mm after 60 days (pl. 1, *D*).

Inoculations were made on shoots of the following species of *Citrus*: *C. sinensis*, Washington Navel and Valencia orange (pl. 1, *K*); *C. grandis*, Marsh grapefruit (pl. 1, *F*); *C. limonia*, the lemon; and *C. aurantium*, the sour orange. Of these, the lemon was the most susceptible, having cankers 20 to 60 mm in radius in 10 days. The sour orange was the most resistant, with small positive lesions 10 to 15 mm in radius in the bark and wood one-half to three-quarter inch in diameter; on older, large wood the results were negative. The sweet orange (Washington Navel and Valencia varieties) was next to the lemon in susceptibility, and had developed much gum and diseased areas of 40 to 70 mm in radius in 15 days. Marsh grapefruit shoots were readily infected, developing cankers 70 mm in radius in 15 days. The fruits of all the species, including lemon, Marsh grapefruit, and Valencia, and Washington Navel were readily infected when mycelium of the fungus was placed in a wound or on the stem end of fruits when they were kept in a moist chamber.

With the walnut strain, 25 inoculations on *Persea americana*, the avocado (table 2), developed small-sized lesions having a 2 to 5 mm radius. With the avocado strain, 11 inoculations agreed in size with those given above, while 10 other inoculations with the avocado strain were larger and produced diseased areas 10 to 50 mm in radius (pl. 1, *A* and *G*).

Fruits of avocado of various degrees of maturity were inoculated with *Botryosphaeria ribis* without being removed from the tree. Young fruits 1 to 1½ inches in diameter, when inoculated through a wound made by a 4-mm cork borer, developed spots having a radius of 4 to 5 mm (pl. 1, *G*) in 12 days. Other inoculations made in the

same way and on the same kind of avocado fruit showed no invasion of the tissue. Fruit of a nearly mature, green-fleshed Guatemalan avocado, inoculated through a wound made by a cork borer, developed an infected spot with a radius of 15 to 20 mm in 7 days. Young fruits of Puebla and Blake avocado inoculated without wounds by placing the mycelium growing in agar on the surface and covering it with nurseryman's tape, gave definite spots 8 to 15 mm in radius in 6 to 10 days. Diseased areas with a radius of 30 to 35 mm were also produced in a Guatemalan fruit when inoculated by placing mycelium on the surface. These results are not surprising since the avocado fruit has numerous natural openings or lenticels through which the mycelium might readily pass.

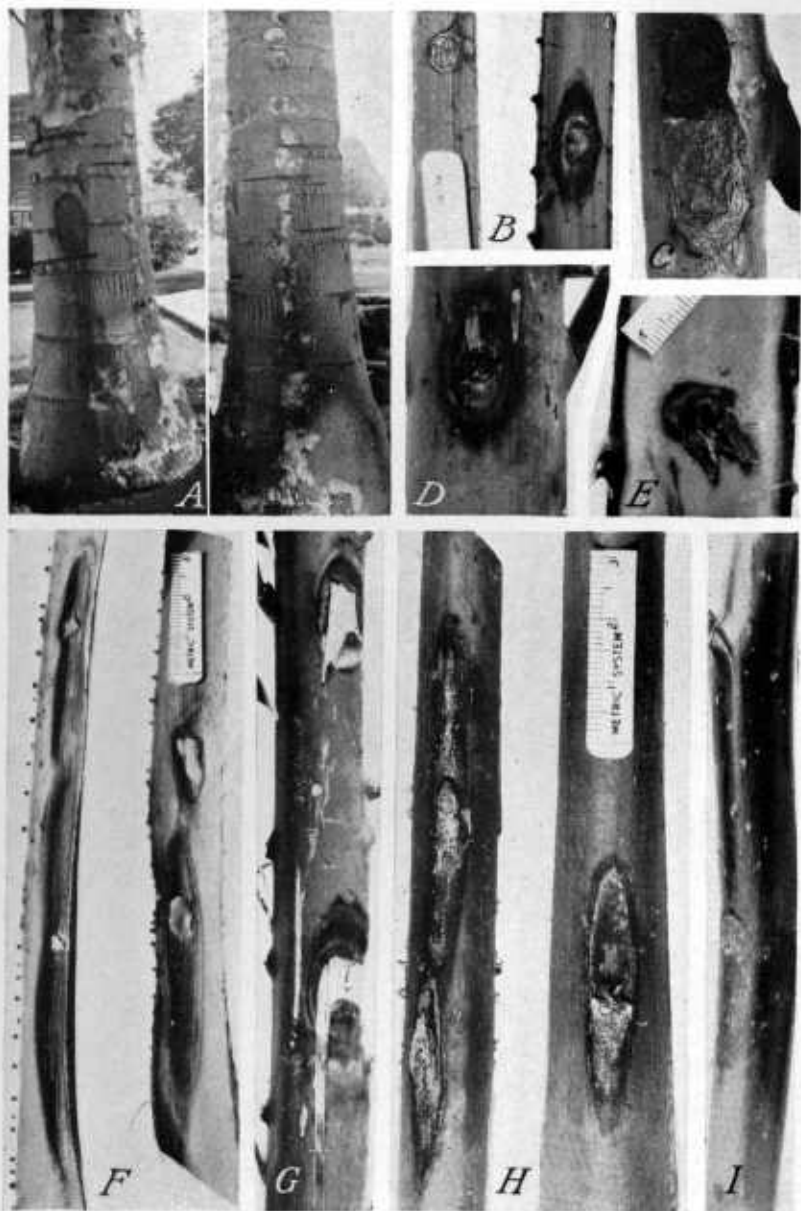
TABLE 2.—Inoculations through wound (unless otherwise indicated) in stems and fruit of *Persea americana* (avocado) with cultures of *Botryosphaeria ribis*

Tissue or kind of fruit inoculated	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	Number	Number	Millimeters
Shoots.....	Walnut.....	18-45	19	15	2-5
Do.....	do.....	18-45	3	3	5-10
Do.....	Avocado.....	18-22	11	11	2-5
Do.....	do.....	25	5	5	5-35
Do.....	Citrus.....	25-33	8	7	20-25
Do.....	Palm.....	25	1	1	5
Guatemalan fruit.....	Walnut.....	7	3	3	20-25
Do.....	Avocado.....	7	3	3	15-20
Do. <sup>1</sup> .....	Walnut.....	7	3	3	18-15
Puebla fruit.....	do.....	7	2	2	15-12
Do.....	Avocado.....	6	2	2	4-5
Blake fruit <sup>1</sup> .....	Walnut.....	6	4	4	8-10
Do.....	Avocado.....	6	1	1	10-15
Do.....	Citrus.....	12	1	1	5

<sup>1</sup> Fruit inoculated while attached to tree by placing mycelium in contact with unwounded surface and covering with nurseryman's tape.

Successful inoculations through wounds with a culture of *Botryosphaeria ribis* isolated from *Cocos plumosa* were made in the following fruits: Apple, lemon, olive, and tomato. Inoculations on woody stems of hosts that had proved to be susceptible to the avocado, citrus, and walnut strains of *B. ribis* were also infected when inoculated with the palm strain of *B. ribis*, and developed into positive lesions that were similar in size, shape, and other characteristics to those made by the other three strains of *B. ribis*. The results from these inoculations (the number of positive results and the radius in millimeters) are given in tables 1 to 4. The inoculations of Palmaceae (pl. 3, B-F) with avocado, citrus, palm, and walnut strains are listed in table 3, and were made during December and January, which during this season were not excessively rainy. The amount of rain falling at Riverside, Calif., during December 1933, was 1.66 inches, and during January 1.01 inches.





NATURAL LESIONS ON *COCOS PLUMOSA* AND INOCULATIONS WITH THE PALM AND CITRUS STRAINS OF *BOTRYOSPHAERIA RIBIS*.

A, Two views of *Cocos plumosa* having natural lesions caused by *Botryosphaeria ribis*, with surface of palm cut away in some lesions to show the blackened tissue. B, *Cocos bonnetii* showing control and lesion formed from inoculation with palm organism, photographed after 5 months. C, Lesion on apple, inoculated with palm organism, showing pycnia that have formed, photographed after about 6 months. D, Lesion on petiole of *Phoenix canariensis* after 60 days. E, Petiole of *Washingtonia filifera* lesion after 60 days. F, Inoculations on *Erythra edulis*, two lesions coalesced at left having been caused by the palm strain of *Botryosphaeria ribis*, the lesion at the right having been caused by the citrus strain. G, Lesion on petioles of date palm, *Phoenix dactylifera*, after 60 days. H, Inoculations on *Cocos plumosa*, showing pycnia that have been formed, photographed after 60 days. I, Artificial lesion on *Juglans regia* caused by palm organism after 35 days.

TABLE 3.—Inoculations through wounds in the petioles of the Palmaceae with cultures of *Botryosphaeria ribis*

[Results after 60 days]

Hosts inoculated <sup>1</sup>	Source of culture	Inoculations	Positive results	Radius of infected area
		Number	Number	Millimeters
* <i>Cocos bonnetti</i> .....	Palm .....	3	1	10
Do. ....	Walnut .....	4	3	5-10
Do. ....	Citrus .....	2	1	10
* <i>C. plumosa</i> .....	Palm .....	8	8	5-30
Do. ....	Walnut .....	5	3	5-120 by 5
Do. ....	Avocado .....	2	0	2
Do. ....	Citrus .....	4	0	2
* <i>Erythea edulis</i> .....	Palm .....	4	4	20-280 by 5
Do. ....	Walnut .....	2	2	10
Do. ....	Citrus .....	3	2	5-10
Do. ....	Avocado .....	1	1	5
* <i>Phoenix canariensis</i> .....	Palm .....	3	3	5-20
Do. ....	Walnut .....	2	2	10-300
Do. ....	Citrus .....	2	2	5
* <i>P. dactylifera</i> .....	Palm .....	3	3	3-5
Do. ....	Walnut .....	3	3	8-10
Do. ....	Citrus .....	2	2	5-10
* <i>Washingtonia filifera</i> .....	Palm .....	2	1	10
Do. ....	Walnut .....	4	3	5-130 by 4
Do. ....	Citrus .....	3	2	5-10

<sup>1</sup> Species that have not been reported so far as the author knows in literature as hosts of *Botryosphaeria ribis* are marked with an asterisk (\*).

<sup>2</sup> Negative.

TABLE 4.—Miscellaneous species inoculated with *Botryosphaeria ribis*

Species inoculated <sup>1</sup>	Source of culture	Period of incubation	Inoculations	Positive results	Radius of infected area
		Days	Number	Number	Millimeters
* <i>Annona cherimola</i> (cherimoya) .....	Walnut .....	15	7	7	25-55
Do. ....	Avocado .....	15	6	6	5-25
* <i>Castanea</i> sp. ....	Citrus .....	33	3	2	5-10
Do. ....	Walnut .....	23	3	3	20-45
* <i>Carissa grandiflora</i> .....	Avocado .....	17	3	2	10-20
* <i>Cerantonia siliqua</i> (carob) .....	Walnut .....	22	3	3	25-35
Do. ....	Avocado .....	22	3	3	5-25
* <i>Chionanthus virginica</i> .....	Walnut .....	16	3	3	10-20
Do. ....	Citrus .....	30	2	0	-----
* <i>Corylus avellana</i> .....	Walnut .....	20	5	2	40-60
* <i>Cupressus macrocarpa</i> .....	do. ....	16	3	3	30-45
* <i>Diospyros kaki</i> .....	do. ....	25	4	3	5-10
<i>D. lotus</i> .....	Avocado .....	30	5	3	5-15
Do. ....	Palm .....	25	3	3	5-10
<i>Eucalyptus</i> sp. ....	Walnut .....	10	3	3	50-65
Do. ....	Palm .....	25	3	2	60-70
* <i>Feijoa sellowiana</i> .....	Walnut .....	45	5	4	20-40
* <i>Ficus</i> (Kadota fig) .....	do. ....	18	3	3	3-10
* <i>Frazinus ornus</i> .....	do. ....	25	3	3	15-20
Do. ....	Avocado .....	25	3	2	2-10
* <i>Juniperus procera</i> .....	Walnut .....	25	3	3	5-35
* <i>Morus nigra</i> .....	do. ....	22	2	2	5-10
* <i>Olea europaea</i> .....	do. ....	10	2	3	10-25
Do. ....	Avocado .....	30	6	6	10-20
Do. ....	Palm .....	25	2	2	5-7
* <i>Platanus racemosa</i> .....	Walnut .....	16	3	3	5-35
Do. ....	Citrus .....	27	3	2	5-10
* <i>Populus fremonti</i> .....	Walnut .....	47	6	2	30-35
<i>Psidium guajava</i> .....	do. ....	33	6	6	15-70
Do. ....	Avocado .....	18	6	3	15-40
Do. ....	Palm .....	25	3	3	35-100
* <i>Quercus lobata</i> .....	Walnut .....	16	3	3	5-30
<i>Ribes vulgare</i> .....	do. ....	90	5	2	10-20
Do. ....	Citrus .....	90	2	0	-----
Do. ....	Avocado .....	90	3	0	-----
<i>Salix nigra</i> .....	Walnut .....	25	10	10	20-40
Do. ....	Citrus .....	33	2	2	10-25
Do. ....	Avocado .....	90	2	0	-----
* <i>Sapium sebiferum</i> .....	Walnut .....	45	3	3	15-30
* <i>Ulmus parvifolia</i> .....	do. ....	16	3	3	10-20
<i>Vitis vinifera</i> .....	do. ....	33	3	3	5-6

<sup>1</sup> Species that have not been reported so far as the author knows in literature as hosts of *Botryosphaeria ribis* are marked with an asterisk (\*).

The results of inoculation on 20 species of miscellaneous plants are summarized in table 4. They include *Castanea* sp., chestnut (pl. 1, *H*); *Corylus avellana*, filbert; *Diospyros kaki* and *D. lotus*, persimmon; *Olea europaea*, olive; *Psidium guajava*, lemon guava; *Populus fremonti*, cottonwood; *Platanus racemosa*, sycamore; *Salix nigra* var. *vallicola*, willow; *Ulmus parvifolia*, elm; *Quercus lobata*, oak; and *Annona cherimola* (pl. 1, *J*).

The diseased areas on the olive (pl. 1, *L*), with the walnut strain had a radius of 10 to 25 mm in 10 days; those on the lemon guava a 50 to 70 mm radius in 15 days; those on *Platanus racemosa* a 10 to 40 mm radius after 16 days; those on *Quercus lobata* a 15 to 30 mm radius in 15 days; those on *Corylus avellana* a 5 to 35 mm radius in 16 days, increasing in size until they were 40 to 50 mm in radius after 60 days (pl. 1, *B*).

Consistently negative results were secured from six inoculations on each of the following species: *Casimiroa edulis* (sapota), *Zizyphus jujuba*, and *Machadma ternifolia* (Queenland nut).

In the inoculations on woody shoots the bark and wood became invaded. The effects of the fungus were most marked on the wood, which was stained to a greater extent than the bark lesion. The extent of this wood invasion is shown in pl. 1, *C*, in the blackened V-shaped area of a section of a eucalyptus stem. Often the invasion of the organism is shown as a narrow, dark-colored strip in woody tissue that is not visible in the bark.

The controls were usually healed over at the end of the experiment in all the hosts except the Palmaceae, in which there was merely a drying of the margin of the wound. (See pl. 3, *B*, a control for an inoculation on *Cocos bonnetti*.)

The inoculations listed in tables 1, 2, and 4 were made with the palm strain in December 1933 and January 1934, and with all other strains from March to August 1933. This latter period was near the end of the rainy season and during the dry season. As shown by the records of the Citrus Experiment Station, Riverside, Calif., the amount of rain in inches was as follows: March 1933, 0.04; April, 1.28 (three showers of 0.31, 0.40, 0.42, and four light sprinkles); May, 0.30; June, 0.02; July, 0. Diseased areas developed during each of 5 months. The difference in their size and number was not significant. During July the inoculations were chiefly confined to avocado fruit, which was infected while on the tree. The temperatures, while variable, did not appear to be a controlling factor when the mycelium was placed in a wound in the tissue and sealed with nurseryman's tape.

#### REISOLATION OF THE FUNGUS

*Botryosphaeria ribis* was readily reisolated in pure cultures from small pieces of tissue cut from the outer margin of the affected areas of selected inoculations taken from most of the species listed in tables 1 to 4.

#### SUMMARY AND DISCUSSION

*Botryosphaeria ribis* is found in many of the temperate and subtropical countries. Its host range in nature includes at least 34 genera and 20 families of plants. Cultures of the *Dothiorella* stage isolated from *Juglans regia*, *Persea americana*, *Citrus limonia*, and

*Cocos plumosa*, when used in cross inoculations, indicate that these strains of *Dothiorella* are identical. The inoculations described in this paper were made on over 50 species of plants distributed among 39 genera and 20 families. The positive results show lesions 5 to 90 mm in radius.

The experiments show that the mycelium of the *Dothiorella* stage of *Botryosphaeria ribis* can invade the bark and woody tissue of a number of different species of Rosaceae, Juglandaceae, Palmaceae, *Citrus*, and other plants of economic importance. This suggests the possibility that some of the large number of described species of *Botryosphaeria* and of *Dothiorella*, based mainly on host relations may be the same. The evidence shows that with wounds and sufficient inoculum certain hosts are extremely susceptible, while others are apparently more resistant. In nearly all the inoculations, healthy tissue was actually invaded. With more humid conditions, larger lesions might be expected. Under natural conditions, it is probable that many of these slightly susceptible hosts would only rarely, if ever, become infected because of the lack of sufficient inoculum to break down the initial resistance.

Relatively few inoculations were made on the different hosts, and a fairly high percentage of positive results were secured. The number of inoculations, however, was too small to give any reliable evidence of the species resistant to the different California strains of *Botryosphaeria ribis*. The experiments were planned to determine the pathogenicity of the organism on different hosts rather than to show plant resistance or even to test whether it is active on some plants and not on others. Often some inoculations were very active, and others on the same branch made under the same conditions were negative. The negative results in the tables probably have no significance.

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