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CONTENTS

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C. E. YARWOOD

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ONION DOWNY MILDEW[1]

C. E. YARWOOD[2]

INTRODUCTION

DOWNY MILDEW of onion, caused by *Peronospora destructor* Berk., is the most important disease of the onion seed crop in California. It is serious on onions grown for bulbs and greens not only here but also in other onion-growing regions throughout the world. Though the disease probably occurs in most regions every year, severe losses are rather sporadic, as is the case with many diseases caused by downy mildews. Most previous attempts to devise control methods for the disease have been unsuccessful. The present study was started in 1935 and is devoted to various biological aspects of the disease and to its control. The work was done in the greenhouse in Berkeley unless otherwise mentioned. The principal literature concerning onion mildew is briefly reviewed, more attention being given to the controversial aspects. Most field observations were made on onions grown for seed, and generalizations in this paper refer to and are based on the California seed crop.

California grows from about 1,000 to 7,000 acres of onions for seed annually (*72*)[3] with a production of perhaps 300,000 to 1,500,000 pounds and a value of perhaps $300,000 to $1,000,000 (no official estimates available). From 1918 to 1929 (*72*) California produced about 95 per cent of the total onion seed for the United States. Since then, partly because of destructive onion-mildew epidemics in California, some of the seed industry has been moved to Oregon and Idaho. The California bulb crop, varying from about 5,000 to 10,000 acres, produces about 1,000,000 to 1,500,000 sacks at a value of about $1,000,000 to $1,800,000 (*7*). California produced about 9 per cent of the total United States crop of onion bulbs during 1935 to 1940. By counties, in approximate order of impor-

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[3] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

tance, California onion seed is produced principally in Sacramento, Santa Clara, San Joaquin, and Sonoma counties, whereas bulbs are grown in quantity in San Joaquin, Kern, Los Angeles, Riverside, Yolo, Stanislaus, Solano, and Monterey counties (*8*). In Kern, Los Angeles, and Riverside counties, onion mildew has been observed in destructive amounts but has attracted less attention than in the other districts mentioned.

In California, onion seed is harvested in July and August on plants grown from mature dormant bulbs which were set in the ground the previous fall and winter. The seed-producing plants make most of their vegetative growth in the spring months, which are during the rainy season, and produce their flowers and mature their seed in the semiarid summer weather that follows. This coincidence of the vegetative-growth period with the cool, rainy season, while apparently favorable for onion growth and for high yields (*28*) in seasons of relative freedom from mildew, may be associated with the destructive onion-mildew epidemics that occasionally occur.

NAME, HISTORY, AND RANGE OF THE DISEASE

Onion downy mildew has also been called mildew, mold, blight, white blast, and rust, but the name downy mildew is preferred for mycological and symptomatological reasons.

Onion downy mildew was first reported in England in 1841 by Berkeley (*1*), but no information concerning its importance was presented at that time. Since then it has been studied intensively in the Bermuda Islands by Shipley (*60*); in New York by Whetzel (*76*), Cook (*12*), and Newhall (*50*); in Ireland by Murphy and McKay (*44*), and McKay (*36*); in Russia by Katterfeld (*31*); and in California by Jones, Porter, and Leach (*29*). In addition to the regions already mentioned, the disease is present in Asia (*10*), Africa (*73*), Australia (*51*), New Zealand (*32*), and Argentina in South America (*34*). With the possible exception of limited localities of unfavorable environment for the disease such as Texas (*65*), onion downy mildew is now considered general in its distribution.

ECONOMIC IMPORTANCE

Nature of Losses.—Onion downy mildew is important because of the large reductions in yield caused by the disease in epidemic seasons. With onions for greens, the yield reduction is direct—the injury to and killing of the leaves caused by mildew infection reduces the yield and salability of the product. Occasionally plants are killed but this is apparently rare. With onions for bulbs, the loss is indirect—mildew injury to the leaves reduces the yield and quality of bulbs. According to Murphy and

McKay (*44*), bulbs from an infected crop are more spongy in character and of poorer keeping quality, and systemically infected bulbs sprout prematurely in storage.

Downy-mildew infection on the onion crop for seed is the most serious aspect of the disease in California. Thaxter (*66*) reported that in Connecticut only seed onions were injured and that bulb onions beside infected seed onions were not infected in a single instance. Trelease (*69*), on the other hand, indicated that in Wisconsin the disease was more serious on young plants.

The reason onion mildew is more serious on the seed crop than on the bulb crop presumably is that the seed plants are exposed to infection for a longer period, and also that the seedstalks represent the last main growth product of the plant. If the seedstalks are injured after the leaves have already been injured or destroyed no seedstalks, or very few more, are formed by the plant in an apparent effort at recovery, whereas if the leaves of plants for bulbs are destroyed new leaves may continue to be formed by the uninjured growing point.

The leaves of well-established plants started from bulbs and grown for seed are apparently of little importance in determining the seed-yielding capacity of those plants. Jones, Porter and Leach (*29*) report the use of leaf pruning as a method of reducing the spread of mildew. While they give no evidence that the leaf pruning did actually reduce the incidence of mildew, they report that a good crop of seed was produced by the treated plants. To secure further information on the effect of leaf pruning, a plot of Italian Red onions growing in Berkeley was subjected to various amounts of leaf pruning. The treatments and results are given in table 1. On September 22, 1937, 10 bulbs were planted with their tops at about the soil level in each of 27 plots. Five plots were left untreated as controls, and on the others the leaves were removed at stated periods. Before seedstalks were formed, leaf pruning was performed by cutting off the leaves about 2 inches above the soil line; after seedstalks were formed, leaves were removed by cutting them off where they clasped the seedstalk or the neck below the seedstalk. Records kept of the number of plants, number of leaves, and length of leaves for some of the plots indicate that onion plants possess a remarkable power to recover after severe leaf pruning. With the exception of the plots which were defoliated four times or more, no marked stunting of the plants was apparent as a result of these treatments. No downy mildew was found in the plots, but during the summer of 1938 many of the plants became infected with *Botrytis allii* M. T. Munn, and some of the seedstalks had been destroyed by *Botrytis* before the August 5 readings were taken. Because of *Botrytis* infection it was not considered worth while

TABLE 1

EFFECT OF LEAF PRUNING ON GROWTH AND PRODUCTIVITY OF ITALIAN RED ONIONS PLANTED SEPTEMBER 22, 1937, BERKELEY

Dates of leaf pruning	Plots	Final plants per plot on Aug. 5*	Leaves per plant							Length of leaves					Seed-stalks per plot on Aug. 5
			On Nov. 19†	On Dec. 8	On Jan. 21	On Mar. 4	On Apr. 15	On May 9	On May 24	On Nov. 19†	On Dec. 8	On Jan. 21	On Mar. 4	On Apr. 15	
	number	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*inch*	*inch*	*inch*	*inch*	*inch*	*number*
None, control	5	8	21	24	27	30	24	..	..	13	18	17	18	15	15
Nov. 19	1	9	..	..	..	..	..	..	..	..	..	..	..	..	20
Mar. 4	1	8	..	..	..	..	..	..	..	..	..	..	..	..	18
April 15	4	8	..	..	..	..	..	..	..	..	..	..	..	..	17
May 9	1	10	..	..	..	..	..	19	..	..	..	..	..	..	23
May 24	1	8	..	..	..	..	..	..	22	..	..	..	..	..	22
Nov. 19, Dec. 8	1	10	..	..	..	..	..	..	..	..	..	..	..	..	21
Jan. 21, May 4	1	7	..	..	..	15	13	..	..	..	..	..	12	12	18
Jan. 21, April 15	1	6	..	..	..	..	15	..	..	..	..	..	..	12	7
April 15, May 9	1	6	..	..	..	..	..	8	..	..	..	..	..	..	11
Jan. 21, May 24	2	10	..	..	..	..	..	..	15	..	..	..	..	..	25
Nov. 19, Dec. 8, Jan. 21	1	6	..	..	..	..	..	..	..	..	..	..	..	..	21
Jan. 21, Mar. 4, May 9	1	5	..	..	..	..	..	6	..	..	..	..	..	..	9
Jan. 21, April 15, May 24	1	8	..	..	..	..	..	..	9	..	..	..	..	..	17
Nov. 19, Dec. 8, Jan. 21, Mar. 4	3	6	..	..	..	..	..	..	..	..	..	..	..	..	10
Jan. 21, Mar. 4, April 15, May 9	1	8	..	..	..	..	..	6	..	..	..	..	..	..	9
Nov. 19, Dec. 8, Jan. 21, Mar. 4, April 15, May 9	1	5	..	10	13	14	12	7	..	..	11	12	11	11	10

* 10 bulbs were planted to each plot.
† 1 control plot only.

to record the seed yield of these plots. In my opinion, however, the results from this experiment corroborate the opinion of Jones, Porter, and Leach (*29*) that leaf pruning does not seriously interfere with seed production. As to whether such treatments will reduce the severity of downy mildew, apparently no information is available, but in cases of severe leaf infection the removal of the leaves would certainly reduce the amount of inoculum.

It is believed that onion mildew may reduce the quality as well as the yield of seed. The large numbers of shriveled seeds in heads on heavily mildewed seedstalks is direct evidence of this. In one of the procedures of cleaning onion seed, the seed is immersed in water, and the light shriveled seeds are floated off and discarded, while the heavier seed that settles to the bottom is saved. To determine if seed from heavily mildewed plants is different in its germination capacity from that of less severely infected plants, seed from the 1937 Milpitas spray plot (table 27) was subjected to a germination test. Before the seed was cleaned by immersion in water, the average percentage germination of seed from 4 control plots and from 4 plots sprayed weekly was 93 and 96 per cent, respectively; after the seed was cleaned it was 83 and 85 per cent, respectively.[4] On the basis of this test there was little difference in the germination capacity of seed from heavily mildewed and from less severely mildewed plants, but these results may be atypical.

Another matter of possible importance in considering the nature of injury from onion mildew is sporulation injury. In tests reported in more detail elsewhere (*85*), the green weight of infected onion leaf tissues on which sporulation occurred averaged only 48 per cent of that of infected tissues on which sporulation was prevented. This sporulation injury was considered in part due to the transfer of food materials from host to fungus, and could not be ascribed to respiration or transpiration. The amount of cardon dioxide produced per gram dry weight of leaf tissues per hour averaged 1.29 mg for healthy leaves, 2.01 mg for mildewed nonsporulating leaves, and 1.80 mg for mildewed sporulating leaves. The respiration of sporulating mildewed tissues was thus 10 per cent less than that of nonsporulating mildewed tissues, and 40 per cent greater than that of healthy tissues; that of nonsporulating mildewed tissues was 56 per cent greater than that of healthy tissues.

Amount of Losses.—Losses from onion mildew in individual field plantings vary from none to total failure. According to Jones, Porter, and Leach (*29*), the loss due to mildew infection in the California seed crop from 1920 to 1938 has varied from 0 to 70 per cent in different years. I have seen individual fields of onions for greens, bulbs, and seed

[4] Data supplied by G. W. Scott of the Associated Seed Growers, Inc.

so severely injured that the crop was not harvested. No satisfactory data from which onion-mildew severity and onion yield might be correlated over a period of years are available, but it is interesting that in 1939, when onion mildew was of little importance in California (where most of the United States onion seed is produced), the United States yield per acre of onion seed was 297 pounds, while in 1940 when mildew was severe in California the yield of onion seed for the United States was only 173 pounds per acre (*71*). Further data on the effect of downy mildew on yields of onion seed are given in the report of field trials of fungicides (tables 25 to 32).

In greenhouse tests, downy-mildew infection slowly kills individual leaves, stunts the plants, and occasionally kills them. To determine the amount of injury from onion mildew under greenhouse conditions, pots of seedlings varying in different tests from 15 to 35 days in age and with 10 to 50 seedlings per pot were inoculated, appropriate controls were maintained, and the green weight of the tops of inoculated and control plants were compared after incubation periods of 21 to 50 days. Only tests were used in which a high percentage of infection occurred in the inoculated plants, and several tests during the heat of the summer were discarded because of low infection. In cases where high infection occurred, there seemed to be little difference in the amount of injury from infection between trials at different seasons or between trials with incubation periods varying from 21 to 50 days from inoculation to harvest. In 16 tests in all seasons from February, 1936, to March, 1938, with an average of 5 inoculated and 5 control pots of plants in each test and with the green weight of the controls varying from 1.6 to 11.0 grams per pot and from 0.21 to 0.48 gram per plant, the green weight of the inoculated plants varied from 29 to 85 per cent and averaged 50 per cent of the controls. In these tests not all of the inoculated plants were infected, so the weight of plants actually infected would be somewhat less and the reduction in green weight due to infection would be somewhat more. Further details on the effect of mildew infection on the yield of greenhouse onions are given in table 24.

The many systemically infected plants in these tests were more severely stunted than the plants only infected locally. When weighed 40 days after inoculation, the systemically infected plants in 2 tests of 10 pots each averaged 0.25 gram per plant and the locally infected plants in the same pots 0.64 gram. Systemic infection also greatly reduces the yield and usually finally kills plants grown from bulbs (see figs. 1 and 2). In one greenhouse test, the tops of 12 systemically infected plants averaged 8.2 grams per plant 36 days after inoculation, whereas tops of 15 comparable healthy plants averaged 12.8 grams.

HOST RANGE

Peronospora destructor has been reported on the following hosts with the following records of its occurrence:

Host	Authority
Allium, species not recorded, but presumably *A. Cepa*	Berkeley, 1841 (*1*)
Welsh onion, *A. fistulosum*	Schleiden, 1846 (*58*)
Shallot, *A. ascalonicum*	Rhitzema Bos, 1898 (*55*)
A. nigrum	Scalia, 1900 (*57*)
Leek, *A. Porrum*	Schoyen, 1901 (*59*)
A. pistulosum [*A. fistulosum?*]	Yoshino, 1905 (*86*)
A. ursinum	Massee and Crossland, 1905 (*40*)
A. oleraceum	Treboux, 1913 (*68*)
Garlic, *A. sativum*	Zimmerman, 1914 (*87*)
Multiplier onion, *A. Cepa* var. *multiplicans*	Murphy and McKay, 1926 (*44*)
Egyptian onion, *A. Cepa* var. *bulbifera*	Murphy and McKay, 1926 (*44*)
Chive, *A. Schoenoprasum*	Cook, 1932 (*12*)

In a field plot at Berkeley in 1936, downy mildew was severe on several varieties of common onion and on one variety of Welsh onion, but no mildew was found on *Allium angulosum, A. cillicium, A. decipiens, A. flookei, A. giganteum, A. hirtifolium, A montanum, A. moschatum, A. odorum, A. ophicorda, A. pallens* (so listed on tag, perhaps *A. paniculatum*), *A. paradoscum, A. pyrenaicum, A. sativum,* and *A. suaveolens.*

Varietal Susceptibility.—All commercial varieties of the common onion, *Allium Cepa,* are believed to be susceptible to downy mildew, though some varieties are more severely injured than others, and the development by hybridization of varieties with a high degree of resistance is under way (*29*). The mildew susceptibility of several commercial onion varieties grown for seed and for bulbs was studied in field plantings at Davis, Berkeley, and Milpitas, and in greenhouse plantings at Berkeley. As there are several methods by which mildew incidence can be determined and varietal susceptibility rated, this matter merits some discussion here.

Three methods of determining mildew incidence are: (1) microscopic observation of tissues for characteristic mycelium, haustoria, and oöspores, (2) determination by the characteristic symptoms, and (3) determination by presence of sporangiophores and sporangia. Microscopic observation of tissues is useful and essential in doubtful cases, and has been extensively used by Murphy and McKay (*44*), but it is too laborious for most studies. The detection of the disease by symptoms is perhaps the most useful method of diagnosis but is subject to error unless one is well acquainted with the disease. One of the most common injury symptoms on onions is the dying of leaf tips. Although this injury may be

TABLE 2

SUSCEPTIBILITY TO ONION DOWNY MILDEW OF ONION VARIETIES GROWN FOR SEED

Variety	1936 Berkeley plot, seedstalks infected, June 13	1937 Milpitas plot		1938 Milpitas plot					1939 plots		
		Leaves infected, March 23	Severity on seedstalks,* June 18	Leaf tissue injured		Sporulation,* April 6	Severity on seedstalks*		Severity,* Milpitas plot		Severity on leaves,* Davis plot, April 10
				March 23	May 27		May 27	July 5	On leaves, May 5	On seedstalks, May 26	
	per cent	*per cent*	*rating*	*per cent*	*per cent*	*rating*	*rating*	*rating*	*rating*	*rating*	*rating*
Ailsa Craig	14	—†	—	—	—	—	—	—	—	—	—
Australian Brown	—	—	—	26	63	7.1	1.2	6.1	—	—	1
California Early Red	30	84	6.2	—	—	—	—	—	—	—	—
Crystal White Wax	—	—	—	39	100	8.6	9.8	10.0	—	—	—
Danvers Flat	10	—	—	—	—	—	—	—	—	—	—
Early Grano	14	—	—	22	85	4.5	3.7	7.4	8.0	8.0	6
Early Yellow Globe	—	—	—	29	83	6.6	3.9	9.0	8.7	6.0	8
Early Snow	37	—	—	—	—	—	—	—	—	—	—
Earliest Express	42	—	—	—	—	—	—	—	—	—	—
Ebenezer	28	—	—	23	80	5.6	3.6	4.5	5.0	1.3	1
Giant White Italian Tripoli	42	—	—	—	—	—	—	—	—	—	—
Italian Red (inbred)	29	54	1.4	—	—	—	—	—	—	—	—
Italian Red 13–53	0	2	0	—	—	—	—	—	—	—	0
Iviza (F.P.I. 64449)	67	—	—	—	—	—	—	—	—	—	—
Lord Howe Island	42	75	6.9	25	97	7.8	4.3	8.9	—	—	8
Ohio Yellow Globe	12	—	—	—	—	—	—	—	—	—	—
Nebuka	100	—	—	—	—	—	—	—	—	—	—
Prizetaker	23	—	—	—	—	—	—	—	—	—	—
Red Creole	81	—	—	—	—	—	—	—	—	—	—
Red Rocco	—	74	9.5	21	49	7.9	5.8	8.0	—	—	—
Red Wethersfield	—	—	—	38	84	7.2	10.0	10.0	—	—	—
Southport White Globe	53	—	—	27	83	6.7	6.2	8.6	3.8	1.6	—
Southport Yellow Globe	—	—	—	28	95	6.2	5.7	8.3	—	—	—
Stockton Yellow Globe	30	83	5.8	—	—	—	—	—	—	—	—
Utah Sweet Spanish	33	—	—	16	89	4.4	1.8	5.2	—	—	0
White Persian	75	—	—	—	—	—	—	—	—	—	10
White Portugal	20	—	—	27	77	7.8	4.6	8.1	6.6	3.0	—
Yellow Bermuda	90	—	—	34	100	7.6	9.9	10.0	8.7	7.2	—
Yellow Globe Danvers	—	—	—	29	91	5.0	7.2	9.2	5.3	1.8	1
Yellow Strassburg	43	—	—	—	—	—	—	—	—	—	—

* In rating relative injury an arbitrary scale of 0 to 10 was used in which 0 indicated no injury and 10 indicated killing of leaf. Intensity of sporulation was also rated on an arbitrary scale of 0 to 10.

† Dashes indicate data not available.

caused by downy mildew, it is also caused by *Botrytis cinerea* Auct. and other, unknown causes, presumably unfavorable soil or weather conditions associated with or in the absence of downy mildew. The occurrence of downy-mildew sporophores on the surface of living onion leaves is the most reliable index of downy-mildew infection, but unfortunately sporulation does not occur with regularity under field conditions.

There are several methods by which varietal susceptibility can be rated. The determination of the percentage of plants infected is useful in comparing the incidence of systemic infection and in comparing the amount of local infection when the incidence of disease is low, but it is of little value in most field tests such as I have observed, where most of the plants are infected in varying degrees of severity. Murphy and McKay (*44*) determined the percentage of plants killed by the disease in one set of comparisons of varieties, but the killing of the plants has been too rare and too slow under the conditions of my observations to make this a useful method. The method I would consider ideal for making comparisons of the susceptibility of varieties to a disease such as downy mildew, the main economic effect of which is to reduce yield, would be to determine the comparative yield reduction due to mildew in several varieties. However, until a satisfactory way can be devised for maintaining healthy control plants under field conditions favoring mildew development, this method cannot be used.

In this study the methods considered most useful for recording varietal susceptibility in the field have been the determination of the percentage of leaves or seedstalks infected, the estimation of the percentage of the leaf tissue showing injury, and the rating of the relative amount of injury or sporulation on leaves or seedstalks. In estimating percentage of leaf tissues showing injury, an independent estimate was made for each of several plants of each variety, and these values were averaged to secure the value for the variety. In rating the relative injury an arbitrary scale of 0 to 10 was used, in which 0 indicated no injury and 10 indicated killing of the leaf; each of several plants of each variety was rated independently and averaged. The intensity of sporulation was also rated on an arbitrary scale of 0 to 10.

The relative mildew susceptibility in the field of several onion varieties as observed in Davis, Berkeley, and Milpitas by different methods of rating is summarized in table 2. The numbers of plants on which these ratings are based were small (the 1936 plot averaged 9 plants per variety and 2 as the minimum number), but I believe the values of table 2 indicate real differences between varieties, though these values are not so satisfactory as yield records would be. The varieties listed as Italian Red (inbred) and Italian Red 13–53 are selections described by Jones, Porter,

TABLE 3

SUSCEPTIBILITY OF ONION VARIETIES TO DOWNY MILDEW IN GREENHOUSE

Variety	Plants from bulbs inoculated by spraying, November, 1936			Seedling plants inoculated by spraying, April, 1937		Seedling plants inoculated by spraying, March, 1938		Dormant bulbs inoculated hypodermically, October, 1940		
	Leaves per plant	Leaves infected	Sporulation*	Plants	Plants infected	Plants	Plants infected	Plants	Plants with systemic infection	Plants with local infection only
	number	*per cent*	*rating*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*per cent*
Brown 5	—†	—	—	—	—	—	—	10‡	30	0
California Early Red	62	84	5	105	99	—	—	—	—	—
Crystal White Wax	—	—	—	160	94	—	—	—	—	—
Early Grano	—	—	—	—	—	—	—	11	82	0
Ebenezer	—	—	—	100	70	—	—	—	—	—
Italian Red 13–53	80	72	5	—	—	—	—	12	17	25
Italian Red 13–53 × Red 21	—	—	—	—	—	117	84	—	—	—
Italian Red 13–53 × 21–22–1	43	77	6	—	—	—	—	—	—	—
Italian Red 13–53 × Lord Howe Island	—	—	—	—	—	210	78	—	—	—
Italian Red 13–53 × Sweet Spanish	—	—	—	—	—	—	—	11	73	9
Italian Red 13–53 × Australian Brown	—	—	—	—	—	—	—	11	27	36
Lord Howe Island	—	—	—	69	91	185	82	—	—	—
Red 21	—	—	—	—	—	118	90	—	—	—
Stockton Yellow Globe	—	—	—	52	90	—	—	—	—	—
Sweet Spanish	—	—	—	137	97	—	—	—	—	—
White Persian	—	—	—	108	97	—	—	—	—	—
Yellow Bermuda	—	—	—	—	—	—	—	10	100	0

* In rating sporulation an arbitrary scale of 0–10 was used in which 0 indicated no sporulation, and 10 indicated maximum sporulation.

† Dashes indicate data not available.

‡ These bulbs grew very slowly and this may account for the low infection.

and Leach (*29*) and used by them in a breeding program aimed at the development of mildew-resistant onions. Among the susceptible varieties White Persian and Yellow Bermuda are outstanding, and mildew has been so severe as to make seed production of these varieties impractical at Milpitas. For this reason, Yellow Bermuda was an ideal variety for tests of fungicidal control (see tables 25, 29, and 31). Among varieties more tolerant to downy mildew are Australian Brown, Utah Sweet Spanish, and Ebenezer. The relative tolerance to mildew of Yellow Bermuda and Australian Brown may be usefully compared by considering the 1937 spray plot on Australian Brown (table 28) with the 1938 spray plot on Yellow Bermuda (table 29). While those were seasons of approximately equal mildew severity at Milpitas, the yield of untreated Australian Brown in 1937 was greater than the yield of twelve-times sprayed Yellow Bermuda in 1938, even though the disease control from spraying was marked in both years.

Greenhouse tests to determine the relative susceptibility of onion varieties were not extensive and were not highly successful, but 4 tests are reported in table 3. In the test of November, 1936, several bulb plants of each of 3 varieties were inoculated, and the number of leaves infected and the relative intensity of sporulation were compared. No marked differences in these varieties were apparent from this test, though field tests show that Italian Red 13–53 is more resistant than the other strains. In the test of April, 1937, differences between greenhouse seedlings were not marked, though Ebenezer, showing least infection in this test is also somewhat resistant under field conditions. In the test of March, 1938, also, the differences between varieties were small and probably not significant. In the test of October, 1940, bulbs of 6 varieties were inoculated hypodermically with a spore suspension. In this test Early Grano and Yellow Bermuda, known to be highly susceptible under field conditions, showed the highest amount of systemic infection. Italian Red 13–53, known to be highly resistant in the field, showed the lowest amount of systemic infection. The results with Italian Red 13–53 × Sweet Spanish and Italian Red 13–53 × Australian Brown show these to be intermediate in susceptibility, as might be expected from field tests. The results with Australian Brown are probably unreliable because of delayed growth.

RESISTANT VARIETIES

What is perhaps the only effort to develop onions resistant to downy mildew has been reported by Jones, Porter, and Leach (*29*). Jones and his associates isolated 3 strains of onions resistant to downy mildew and have used these in an extensive breeding program aimed at the development of mildew-resistant strains of commercial onions. The results at

present appear promising, and some of these parent strains and hybrids of them have remained resistant to onion mildew and produced high yields under epidemic conditions for the disease at Milpitas, while plantings of several commercial varieties were almost destroyed by mildew (table 2). The ultimate and permanent success of such a program would make unnecessary any other control practices for this disease. In such a program, however, there is always the danger that strains of the organism capable of causing injury to the new varieties will appear.

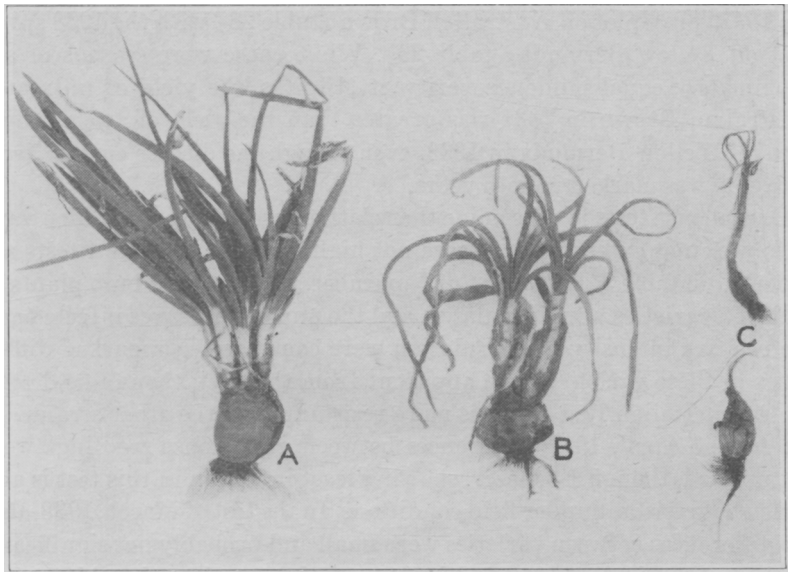

Fig. 1.—Effect of systemic infection with onion downy mildew on plants of Stockton Yellow Globe onions grown for seed at Milpitas. Photographed February 21, 1939. *A*, Typical plant, showing no systemic infection with downy mildew but showing a characteristic killing of the leaf tips, the cause of which was not determined for this material, and some local white infection spots caused by *Botrytis cinerea; B*, systemic infection on a plant that was healthy on November 7, 1938; *C*, plants which showed systemic infection on November 7, 1938.

No extensive studies of the nature of resistance in the onion strains developed by Jones, Porter, and Leach (*29*) have been made. In greenhouse cultures, sprayed water adhered less readily to the leaves of mildew-resistant Italian Red 13–53 plants and to leaves of intermediate susceptibility from white bulbs of an unknown variety than to leaves of the highly susceptible Yellow Bermuda. In the field at Milpitas, however, no marked difference in the retention of rain water or dew by the leaves of several strains with known differences in resistance could be observed.

SYMPTOMS

Assuming that onion downy mildew oversummers as mycelium in dormant bulbs, the first symptoms to be observed on onions grown for seed from bulbs are the pale-green, down-curled, and narrow leaves of the systemically infected plants (figs. 1, 2). On systemically infected plants growing in the greenhouse, two symptoms not previously recorded have been frequently observed. On some leaves whitish diffuse spots (fig.

Fig. 2.—Effect of systemic infection on Red Creole and Yellow Bermuda onions grown in Berkeley. Three plants at left are healthy, 3 plants at right show systemic infection. In each group of 3 plants, the 2 plants on the left are Yellow Bermuda and the single plant on the right is Red Creole. Planted August 25, 1938; photographed October 3, 1938.

3) somewhat similar to lesions caused by *Botrytis cinerea* (fig. 4) appear. These spots differ from *Botrytis* lesions, however, in not being depressed, and in being less necrotic. Whereas *Botrytis* lesions are elongated in the direction of the long axis of the leaf and about twice as long as wide, the lesions associated with systemic downy-mildew infection are not infrequently wider than they are long. That these lesions are not due to an external infection is indicated by the fact that they appeared on plants kept in a dry environment, and that adjacent nonmildewed plants showed no such symptoms. The other unreported symptom associated with systemic infections is the appearance in spots of whitish crystalline-appearing deposits on the surface of systemically infected leaves.

Primary infection, resulting from systemically infected bulbs, and giving rise to the symptoms just described, is rarely observed, whereas secondary, or external, infection, from sporangia produced by primary infections, is responsible for the well-known and frequently described

Fig. 3.—Localized spotting of onion leaves associated with systemic infection with onion downy mildew. Leaf on left is from healthy plant. Three leaves on right are from systemically infected plants grown in the greenhouse from bulbs inoculated October 26, 1940. Photographed December 2, 1940.

symptoms of onion mildew on leaves and seedstalks. Secondary symptoms consist of oval to cylindric, local lesions usually 3 to 30 cm in length (figs. 5, 6, 7, 8) which appear on leaves and seedstalks. These lesions, always elongated in the direction of the long axis of the leaf, may show no symptoms until after sporulation; they may show as uniformly pale

areas; or they may show as concentric ovals or arcs of slightly chlorotic tissue alternating with tissues of a slightly different shade of green. These large, smooth-margined, oval lesions caused by onion mildew are in marked contrast to the symptoms of many other downy mildews such as

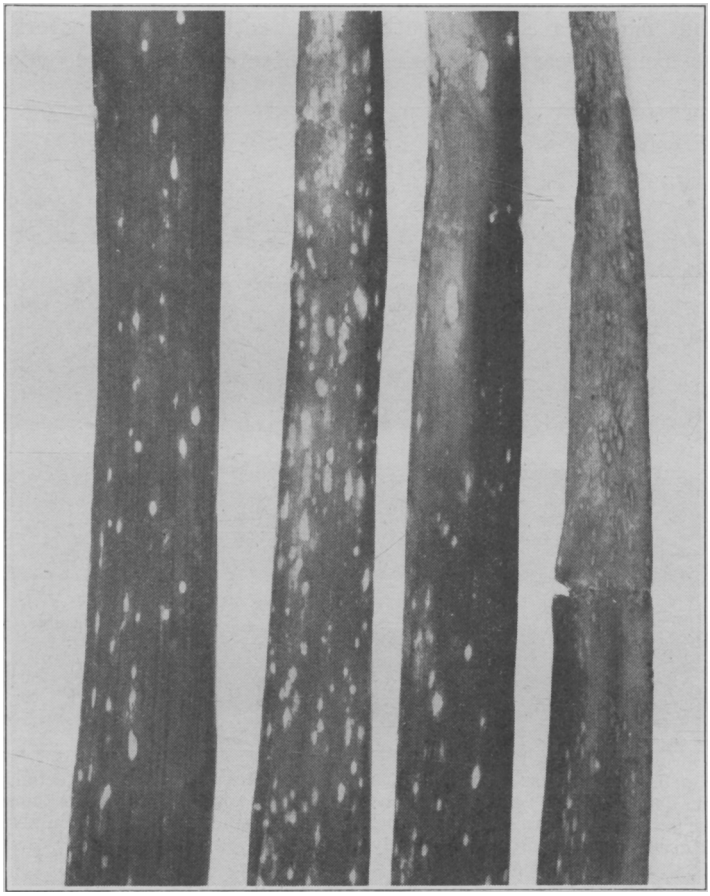

Fig. 4.—White spotting of onion leaves, caused by *Botrytis cinerea*, from plants grown at San Pablo. Leaves from left to right show progressively increasing injury from infection. Photographed February 9, 1940.

those of hop, cucumber, lettuce, and grape, where the lesions on the leaves are usually small, angular, and bounded by the veins. This difference between onion mildew and the other mildews mentioned is believed to be due to the difference in morphology between onion leaves and the leaves of the other hosts mentioned. In onion, the vascular bundles of the leaves and seedstalks are not mechanical or only slightly so (see fig. 11) and

offer little obstruction to the lateral growth of the fungus. The lesions are elongate in the direction of the long axis of the leaf presumably because most of the host cells, other than the palisade parenchyma, are elongated in the same direction.

Infected onion leaf tissue may die without the occurrence of sporulation, but the latter hastens death of infected tissue. The centers of the lesions usually become necrotic first; infection with a secondary in-

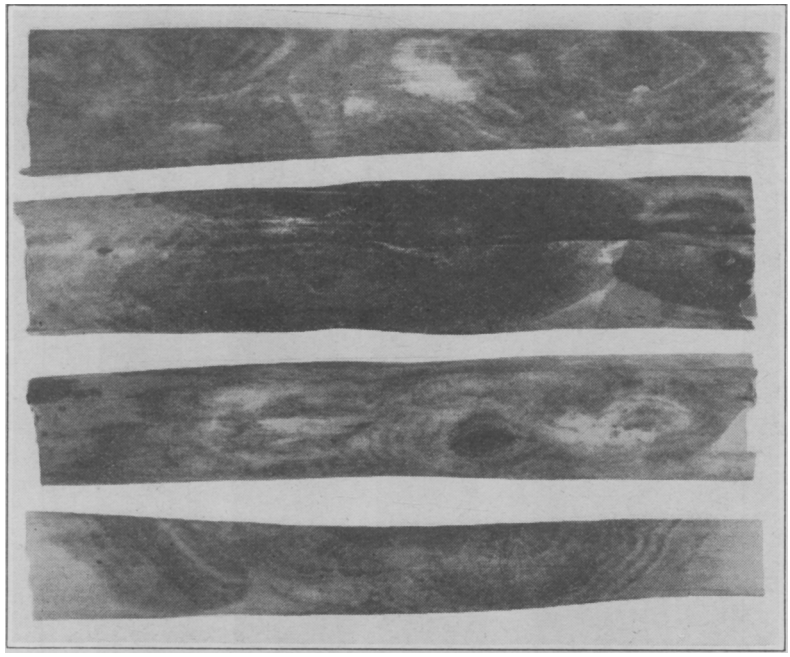

Fig. 5.—*Macrosporium* infection on onion seedstalks previously infected with onion downy mildew; specimens from Cotati. The zonate character is the same basic pattern as shown earlier (see fig. 8) by downy-mildew infection in absence of *Macrosporium*. Photographed July 17, 1940.

vader, *Macrosporium*, usually apparently precedes death of the tissues from mildew infection and is apparently responsible for the rapid killing of tissues already weakened by mildew infection. Early-occurring mildew lesions on leaves or seedstalks generally girdle the organ, and all tissues beyond the lesion may die. In cases of heavy infection, the older outer leaves are progressively killed back to the leaf sheath (fig. 9), while even in the later severe stages of the disease the younger leaves may show only killing of the leaf tips with the base of those central leaves apparently healthy. This type of symptom is a consequence of the manner of growth of the onion. The outer leaves are the older, and after

they have reached a fair length they stop growing, while leaves of intermediate age are still growing, and still younger central leaves are being formed. As the growth of onion leaves is from the base, infected tissues

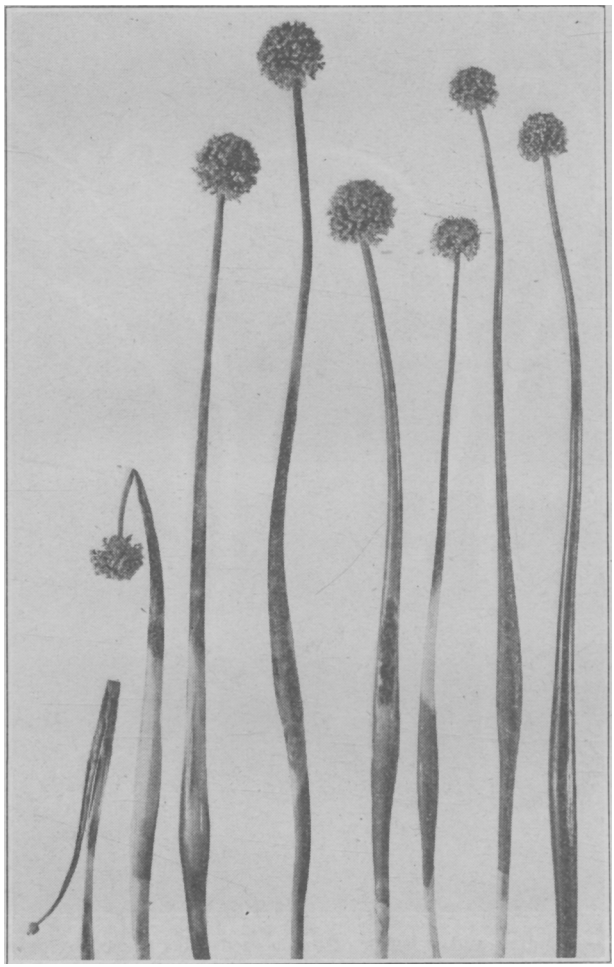

Fig. 6.—Onion-downy-mildew infection of various degrees of severity from complete killing of the seedstalks (extreme left) to healthy seedstalk (extreme right); specimens from Cotati. Photographed July 17, 1940.

on these leaves are being pushed farther away from the base of the plant, and as the infected tissues die a tip-blight condition of the leaves becomes apparent. As the mildew lesion is growing while the leaf is growing, however, the symptoms expressed by the plant may be considered as a balance between several forces, the most important of which are the upward

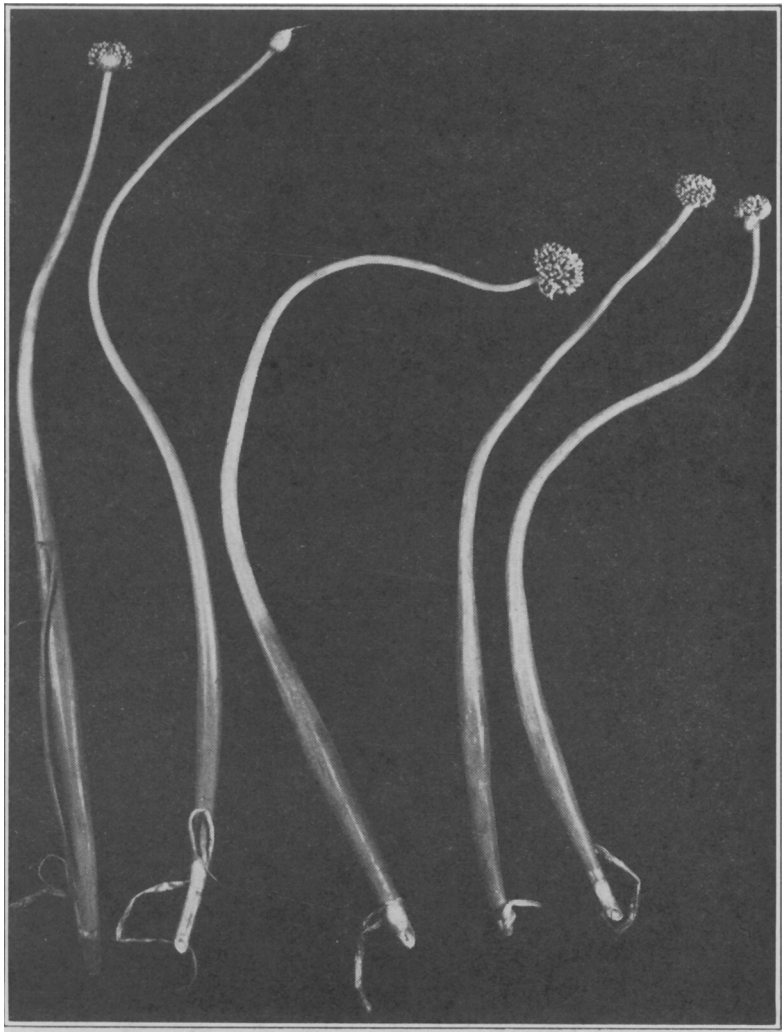

Fig. 7.—Bending and twisting of onion seedstalks caused by onion-downy-mildew infection; specimens from Cotati. Infection on one side of a seedstalk apparently may cause the cells on that side to cease elongating, and thus the stem bends toward the side with the mildew lesion. Later the lesion usually girdles the seedstalk. Photographed June 3, 1941.

growth of the leaves, the downward growth of the mildew fungus, and the number of new secondary infections.

In the field the downward growth of the mycelium and the number of secondary infections are frequently sufficient to kill all the leaves, first the outer leaves and then the inner. Presumably, as the outer leaves are

killed the rate of growth of the inner leaves is decreased because of decreased food supply, and they are thus more subject to complete invasion though perhaps no more susceptible.

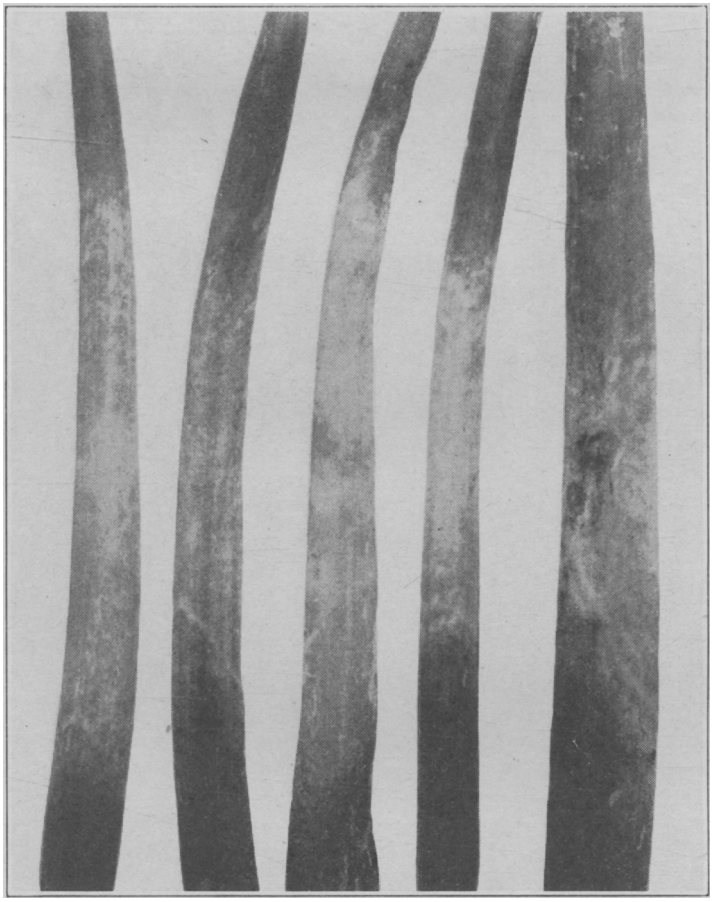

Fig. 8.—Onion leaves with chlorotic areas caused by mildew infection. The leaf at the right shows the zonate character of the chlorotic lesion which is characteristic of many lesions. Photographed May 15, 1941.

In the greenhouse with a temperature at about 20° C, onion leaves of plants grown from bulbs develop rapidly. By marking the point of emergence of leaves from the sheath, onion leaves have been observed to grow as much as 1 inch in 24 hours. When such greenhouse plants are inoculated, all the exposed tissues are usually invaded by the fungus which moves down from the point of inoculation. Outer, nongrowing leaves and the tips of leaves that were exposed at the time of inoculation may

be killed, but so rapid is the growth from below that, in the absence of further secondary infection, the amount of healthy tissue is soon greater than the amount of diseased tissue, and, as new leaves are formed

Fig. 9.—Onion-downy-mildew infection on onions grown for greens, showing the severe tip blight and killing of the lower leaves; from plants grown at Bay Farm Island. Photographed December 15, 1939.

and the older ones are killed back, the plants soon show only healthy living leaves and dead outer leaves. As older outer leaves frequently die in the absence of downy mildew or other known disease, such recovered plants present almost a normal appearance. This process of apparent complete recovery has not been observed in the field. With the exception

of differences apparently determined by rate of leaf growth and amount of secondary infection, as already described (p. 612), leaf symptoms are basically similar in field and greenhouse infections. Seedstalk infection has not been studied to any extent in the greenhouse.

Seedstalks appear somewhat more resistant to infection injury and are less likely to show sporulation than onion leaves. Systemically infected seedstalks are rare, presumably because systemically infected

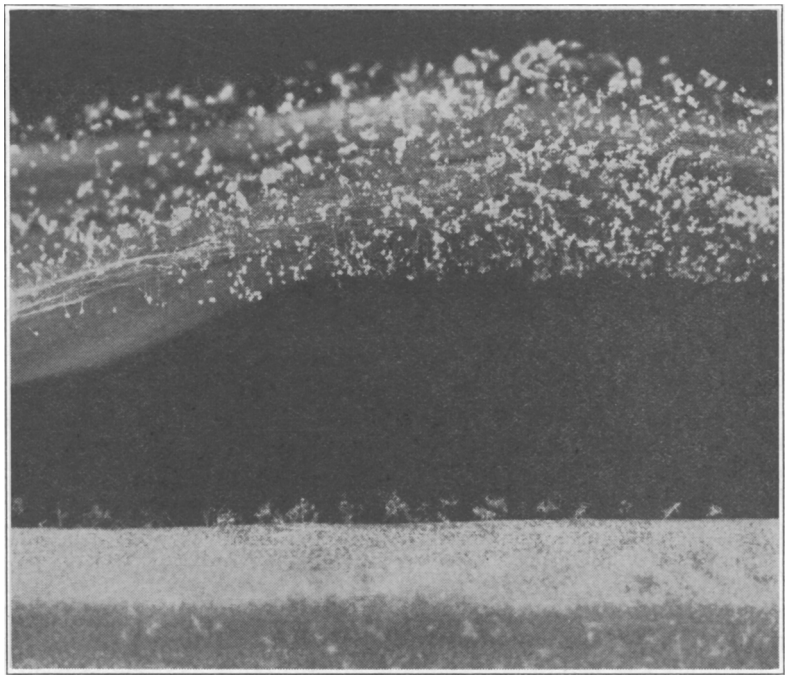

Fig. 10.—Sporulation of *Botrytis cinerea* Auct. (upper) and *Peronospora destructor* Berk. (lower) on same onion leaf (× 8). The *Botrytis* infection is on the killed tip of a leaf which showed infection of onion downy mildew on the lower living portion of the leaf. The sporulation of *P. destructor* is more sparse than usually observed.

plants usually die before seedstalks are formed or the plants are too weak to form them. A common site of local seedstalk infection is a few inches below the head, and infection here and on other portions frequently cause the stalks to bend and curl into various unusual positions (fig. 7). The mildew lesion is usually centered at the inside of the bend, and the bending is apparently caused by the continued linear growth of noninfected tissues while growth is stopped or reduced in the infected tissues. Seedstalks thus affected are usually more rigid than erect, healthy seedstalks. This twisting of the seedstalks is a very striking

symptom on some varieties before there has been much killing of the tissues. Infected areas at the upper end of the seedstalk appear less likely to be invaded by *Macrosporium* than infected areas in the central or lower regions.

Sporulation results in a grayish-violet downy layer of sporangiophores and sporangia of the causative organism (*Peronospora destructor*) on the leaf surface. These sporangiophores and sporangia are the signs of onion downy mildew, and are somewhat similar to, though easily distinguished from, the conidiophores and conidia of *Botrytis cinerea* (fig. 10). Their morphology is described in the next section.

NOMENCLATURE AND DESCRIPTION OF CAUSAL ORGANISM

Peronospora destructor Berk. is a typical downy mildew of the family Peronosporaceae. This binomial with Berkeley as the sole authority has apparently not previously been used and therefore an explanation is in order. Onion downy mildew was first recorded in 1841 by Berkeley (*1*), who illustrated and described the imperfect stage and named it *Botrytis destructor*, though from his description it is obviously a downy mildew, as we now understand this group. In 1846, Schleiden (*58*, p. 38) illustrated an organism on *Allium fistulosum* and referred to it as *B.* (*parasitica* ?), though his illustration is obviously also that of a downy mildew. In 1847, Unger (*70*) gave a Latin description of what appears to be the same organism under the name of *P. Schleideni* Ung. In 1860 Berkeley (*2*, p. 348) indicated that in view of recently acquired knowledge of the oöspores of the group *Peronospora*, the organism he originally described as *B. destructor* should be called *P. destructor* Casp. It is not clear from Berkeley's discussion whether or not he saw the oöspores of the onion-mildew organism, or why he cited Caspary as the authority for his new name. De Bary (*13*) in 1863 refers to the onion-downy-mildew organism as *P. Schleideniana* Unger, though Unger used the spelling *Schleideni*. Worthington Smith (*62*) in 1884 was perhaps the first to record with relative certainty and illustrate the oöspores of the onion-downy-mildew organism.

For the past forty years the name *Peronospora Schleideni* Ung. has been the most widely used binomial for the organism causing onion mildew. There are obvious reasons, however, why it may be considered invalid, and the nomenclature of this organism has been considered in detail by Wilson (*77*), Cook (*12*), and Wakefield and Moore (*74*). Wilson and Cook accept the name *P. destructor* (Berkeley) Caspary on the basis of priority, using Caspary as an authority for the genus, though with reluctance, because Berkeley had used it. Wakefield and

Moore, interpreting article 37 of the *International Rules of Botanical Nomenclature* (*3*) as applying to the Phycomycetes, and believing that Berkeley was not aware of the oöspores of the onion-mildew organism, feel that the name should be *P. Schleideniana* W. G. Smith. In article 37 of the *International Rules,* however, the Phycomycetes are not mentioned, and the Peronosporaceae are usually classified on the basis of their imperfect stages. I agree with Wilson and Cook that the name should be *P. destructor,* but cannot accept their reason—probably an old manuscript name (*77, 12*)—as adequate for including Caspary as the second authority for this name. Even if article 37 of the *International Rules* is considered applicable here, it might still be reasoned that the name should be *P. destructor* Berk. since Berkeley transferred it to the genus *Peronospora* because he believed it possessed an oöspore stage.

The synonomy of *Peronospora destructor* Berkeley is as follows:

Botrytis destructor (Berkeley, 1841) (*1*)
Botrytis (*parasitica* ?) (Schleiden, 1846) (*58*, p. 38)
Peronospora Schleideni (Unger, 1847) (*70*)
P. destructor Casp. (Berkeley, 1860) (*2*)
P. Schleideniana Unger (De Bary, 1863) (*13*)
P. Alliorum (Fuckel, 1870) (*21*)
P. Schleideniana De Bary f. *Cepae* (Thuemen, 1879) (*67*)
P. destructor (Berk.) Caspary (Wilson, 1914) (*77*)
P. Schleideniana W. G. Smith (Wakefield and Moore, 1936) (*74*)

From material I have collected and examined, the morphology of *Peronospora destructor* is as follows (see fig. 11):

Sporangiophores nonseptate, various shades of violet, emerging from stomata, 122 to 820μ in length, 7 to 18μ in diameter at swollen base, tapering to acute sterigmata at tips, two to six times monopodially branched, 3 to 63 sporangia per sporangiophore. Sporangia pyriform to fusiform, attached to sporangiophore by pointed end, 18 to 29μ by 40 to 72μ, thin walled, slightly papillate at proximal end, germinating by 1 to 2 germ tubes from near region of proximal end. Germ tubes penetrate host through stomata and usually form an appressorium over stomatal opening and a substomatal vesicle in substomatal cavity. Mycelium nonseptate, intercellular 4 to 13μ in diameter. Haustoria filamentous, coiled within cells, 1.3 to 5.0μ in diameter. Oögonia 43 to 54μ, oöspores 40 to 44μ.

This description differs from some previous descriptions of the onion-mildew fungus and therefore several controversial aspects of the morphology of the organism will be considered in more detail. I have seen only nonseptate sporangiophores, though septate sporangiophores are illustrated by Smith (*62*) and Massee (*39*). Throughout this paper the terms sporangiophores and sporangia will be used in place of, though synonymous with, the terms conidiophores and conidia of most previous investigators, for reasons given by Fitzpatrick (*20*). The greater

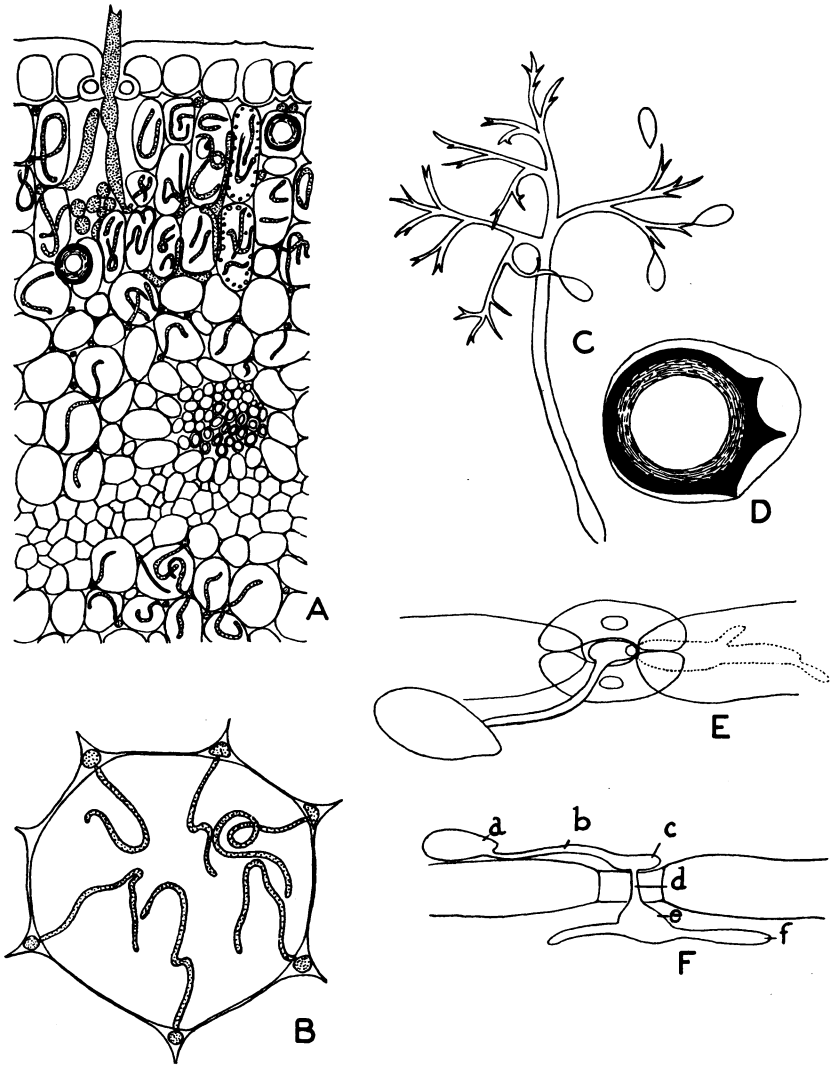

Fig. 11.—*A*, Cross section of onion seedstalk showing mycelium, haustoria, oöspores, and sporangiophores of *Peronospora destructor* Berk. in infected tissues (× 107). *B*, Cross section of parenchyma cell showing intercellular mycelium and intra-cellular haustoria (× 255). *C*, Sporangiophore and sporangia (× 255). *D*, Approximately mature oöspores (× 510). *E*, Stomatal penetration as observed in epidermal strip from inoculated leaf (× 510). *F*, Diagrammatic representation of *E* in radial section (not observed in this manner): *a*, sporangium; *b*, germ tube; *c*, appressorium; *d*, penetration tube; *e*, substomatal vesicle; *f*, infection hypha.

variation in the length of the sporangiophores than previously reported is presumably because the limits recorded in this paper are from temperature and humidity tests, to be presented later (p. 635), and the length of the sporangiophores is greater at higher temperatures and higher relative humidities.

As I have observed them, the sporangiophores of *Peronospora destructor* are monopodially[5] branched, while according to Fitzpatrick (*20*), Saccardo (*56*), and others, the genus *Peronospora* is characterized by dichotomous branching. Saccardo, describing *P. destructor* under the

TABLE 4

COMPARISON OF BRANCHING OF SPORANGIOPHORES OF *Peronospora destructor* AS OBSERVED BY DIFFERENT WORKERS

Authority	Sporangiophores	Average of ultimate branches per sporangiophore	Percentage of ultimate branches on first branch from main axis
	number	*number*	*per cent*
Berkeley, 1841 (*1*)	1	16	37
Schleiden, 1846 (*58*)	1	13	23
Smith, 1884 (*62*)	2	23	35
Shipley, 1887 (*60*)	3	22	21
Whetzel, 1904 (*76*)	2	23	35
Duggar, 1909 (*17*)	1	22	27
Murphy and McKay, 1926 (*44*)	1	109	32
Owens, 1928 (*53*)	1	20	25
Newhall, 1939 (*50*)	63	16	—*
Yarwood, Jan. 9, 1935	8	25	—*
Yarwood, Jan. 24, 1940	8	50	34

* Dashes indicate data not taken.

name *P. Schleideni,* includes the character of dichotomous branching, but the first illustration and description of the fungus by Berkeley (*1*) indicates definite monopodial and alternate branching of the sporangiophores. In later illustrations by Schleiden (*58*), Smith (*62*), Shipley (*60*), Whetzel (*76*), Duggar (*17*), Murphy and McKay (*44*), and Owens (*53*), the branching is distinctly monopodial in most cases. To secure more quantitative information on the branching character of *P. destructor,* measurements were made of material collected by myself as well as from illustrations of previous investigators, and the number of ultimate branches on the first branch from the main axis is expressed as a percentage of the total number of ultimate branches (table 4). If the branching were dichotomous it would be impossible to choose a main axis, and the number of ultimate branches on either of the primary branches should approach 50 per cent of the total. From the calculations

[5] *Monopodial* means possessing a main axis and having secondary side branches; *dichotomous* means with no main axis and with branches of equal size at each division of the main or subsequent stems of the sporangiophore.

of table 4, the maximum number of ultimate branches on the first main branch was 37 per cent of the total (*1*), the minimum was 21 per cent (*60*), and the average was 30 per cent. The illustration chosen arbitrarily by myself (fig. 11) shows 40 per cent of the ultimate branches on the first main branch and is therefore slightly abnormal. If dichotomous branching is an important character of members of the genus *Peronospora,* then onion mildew should not be in this genus.

The number of ultimate branches, or sporangia, per sporangiophore, is an extremely variable character (table 4), and in my tests has varied from 3 (found in one test at 7° C) to 63, while Murphy and McKay (*44*) illustrate a sporangiophore with 109 ultimate branches.

The large pyriform sporangia of *Peronospora destructor* are easily distinguishable from the sporangia of most other downy mildews and of most other fungi, but the shape of the sporangia shows considerable variation. In the illustrations of Schleiden (*58*) and Smith (*62*), the sporangia are ovate to cylindrical with rounded ends. Illustrations by Dudley (*16*) indicate fusiform sporangia, and my cultures, in which sporangia were produced at 22° C, showed predominantly fusiform sporangia, though at lower temperatures they were predominantly pyriform. Sporangia formed in strong artificial light were sometimes constricted in the middle. Duggar (*17*) illustrates the sporangia attached to the sporangiophores by the large rounded end, but in my observation they are always attached at the small pointed end.

Clements and Shear (*11*) indicate that the sporangia of *Peronospora* are not papillate at the tips, and use this character to separate *Peronospora* from *Bremia.* A semblance of a papilla, however, can sometimes be clearly seen at the proximate end of the sporangia of *P. destructor.* The mycelium of *P. destructor* is generally regarded as nonseptate, but McKay (*36*) describes and illustrates septate mycelium arising from germinating oöspores. McKay also describes chlamydosporelike bodies on special branches of the mycelium from germinating oöspores.

Oöspores do not occur with regularity in infected tissues, and the causes underlying their formation have not been determined. Some lesions may show them in abundance, whereas other similar-appearing lesions may show none. I have found them more abundant in seedstalk lesions than in leaves, but they may also occur in abundance in locally and systemically infected leaves. In seedstalks I have seen them most abundant in the palisade region.

The characters most useful in distinguishing *Peronospora destructor* from closely related forms are, in order of merit: the large pyriform sporangia, the filamentous haustoria, the monopodial branching of the sporangiophores, and the germination of the sporangia by germ tubes.

The possibility exists that there are different strains or even species of the onion-downy-mildew organism, and that this explains the large differences in the organism as observed by different investigators. Hiura (*26*) has suggested the existence of strains on the basis of limited host-range studies, but the settling of this point must await comparative cultural studies of various collections of onion mildew.

ATTEMPTS TO CULTURE PERONOSPORA DESTRUCTOR ON ARTIFICIAL MEDIA

L. R. Jones (*30*) reported that attempts to grow the onion-mildew fungus on artificial media were unsuccessful, but he gives no details. McKay (*36*), using oöspores of which the germination had been stimulated by dilute potassium permanganate, reported that the total length of hyphae from oöspores in water varied from 2 to 8 mm in 3 days, and from oöspores on potato dextrose agar plus onion sap varied from 4 to 10 mm in 48 hours. The longest growth recorded by McKay was 15 mm in 6 days. McKay gives only few details of these tests and does not indicate that the potassium permanganate increased the length of the germ tubes apart from its effect in stimulating germination.

I have attempted in 180 tests with an average of 14 plates of different treatments per test to culture *Peronospora destructor* in liquid and agar media, but without success. Many treatments have given stimulation of the growth of the germ tubes, however.

Treatments Used.—In most of these studies, the test materials in solution or suspension were added separately to sterile petri dishes, and 15 cc of melted 1.7 per cent washed plain agar were added and mixed with the test materials. After the poured plates had cooled, they were placed in the bottom of a large galvanized can and seeded lightly by dusting them with fresh sporangia of onion mildew from greenhouse cultures usually at from 10 a.m. to 12 m., and incubated at 16° or 19° C in the dark for 2 to 4 days. Most culture studies were made in the winter and spring months. The cultures in liquid media were handled similarly except that agar was omitted. Sixty-seven different nutrient substances were tested separately and in various combinations in the agar cultures, and 5 in the liquid media. In evaluating the results, 10 germ tubes on each plate were selected at random and measured with an eyepiece micrometer.

The relatively pure test chemicals added to plain agar to determine their effect on the growth of *Peronospora destructor* were as follows: $KMnO_4$, KOH, KNO_3, $KClO_4$, $K_2S_2O_8$, K_2HPO_4, KCl, HCl, HNO_3, H_3BO_3, H_2O_2, MnO_2, $MgSO_4$, Na_2HPO_4, $CaCO_3$, Barnes' mineral nutrient mixture (*54*), microelement mixture A (*27*), microelement

mixture B (*27*), sucrose, dextrose, mannitol, asparagine, *dl*-alanine, cysteine hydrochloride, glycine, glutathion, nucleic acid, tyrosine, hippuric acid, leucine, malonic acid, edestin, tryptamine hydrochloride, choline hydrochloride, salicin, arbutin, tryptophane, heterauxin, indole acetic acid, nicotinic acid, pimelic acid, carotene, riboflavin, wound hormone, vitamin A, vitamin B_1, vitamin C, phenol, potassium pyrogallate, paraffin oil, potassium acid phthalate, and brown sugar.

The extracts tested were of potato, lima bean, yeast, mycelium cultures of *Phytophthora citrophthora* (S. and S.) Leonian, germ-tube cultures of *Peronospora destructor,* mycelium cultures of *Rhizopus nigricans* Ehr., onion leaves, onion bulbs, rabbit liver, rabbit spleen, rabbit blood, rabbit kidney, egg yolk, and orange juice. The above test sources of growth-stimulating substances were mostly extracted in water at room temperatures, but the potato and lima-bean extracts were prepared with heat. Extracts of *Phytophthora, Rhizopus,* and *Peronospora* cultures were made under a variety of temperature conditions and with a variety of extractives. All pure materials and extracts were used in various amounts and combinations and all were used well below any apparent toxic level, as judged from the earlier tests. The pure materials were usually sterilized in solution with heat before use, but the extracts except potato and lima bean were unsterilized and were used immediately or held at 0° C.

With the exception of potassium permanganate, most of the test materials increased the amount of contamination on the plates. Only ordinary precautions were taken to avoid contamination, and contaminants were introduced from the greenhouse with the inoculum, from the laboratory air, and from the test chemicals. A few contaminants were usually present in the cultures, but as growth of *Peronospora destructor* had usually stopped before the contaminants had made any considerable growth, they were not considered of importance in the 4-day period that most of these cultures were kept.

Nature of the Growth.—On plain agar, germination of fresh sporangia usually varied from 30 to 100 per cent, and poor germination of the controls was never a limiting factor in these tests. Usually only 1 germ tube was formed by each sporangium, but occasionally 2 were formed. The germ tubes rarely branched. Growth was mainly on the surface of the agar, but some germ tubes grew down into the agar. Growth continued for about 2 days, and in about 4 days death of the germ tubes was indicated by the disorganized and beaded condition of the protoplasm of the germ tubes. On agar the germ tubes were fairly straight and easily measured, whereas in water they frequently grew in coils and were difficult to measure.

None of the treatments markedly increased the number of sporangia germinating or the number of germ tubes per sporangium, though many increased the branching. Some materials stimulated the formation of short side branches which bore a resemblance to haustoria and which were smaller in diameter than the main germ tubes. Some treatments caused the germ tubes to grow down into the agar to a greater extent than occurred on plain agar.

The most striking effects of the stimulatory treatments, however, were to increase the length and period of viability of the germ tubes. The longest germ tube observed was 3,880μ on a medium consisting of 15 cc water, 1 cc lima-bean decoction, 250 mg agar, 1 mg $KMnO_4$, 2 mg KNO_3, 200 mg sucrose, 0.1 mg glutathion, 0.1 mg vitamin C, and 0.003 mg riboflavin. No strong significance is attached to this mixture for in this trial several other media yielded germ tubes almost as long. The longest period of life of the germ tubes was 6 days, also secured with the above mixture. No septation of the mycelium or formation of chlamydospore-like structures, as described by McKay (*36*), were observed in these cultures.

Variability of Germ-Tube Length.—In 4 tests selected at random, the coefficient of variability of germ-tube length as determined from 10 measurements on plain agar varied from 20 to 33 per cent, with an average of 28 per cent. The variability was about the same on other media. While this variability may be high in absolute terms, it is a great deal less than the variability of the germ tubes from the conidia of several powdery mildews as observed in similar culture tests.

Rate of Growth of Germ Tubes.—Katterfeld (*31*) and Cook (*12*) have reported that the germ tubes of the sporangia of *Peronospora destructor* grew at the rate of about 100μ per hour in water. A similar rate of growth of germ tubes from oöspores may be inferred from data of McKay (*36*). This is a more rapid rate than I have observed in agar cultures. Results of 2 tests are given in table 5. While these tests were not continued to show the time at which growth stopped, the final values for each test reported are about the maximum for the conditions specified, and only in 2 cases in other tests was an average growth of over 1,210μ secured on plain agar. According to these results the maximum rate of growth, occurring between 8 and 27 hours after seeding, was about 50μ per hour, and the average was considerably less.

Amount of Stimulation.—In 74 tests on different days in which the growth on plain agar was compared with the growth on plain agar to which various substances had been added, the minimum average growth of 10 random germ tubes on 1.7 per cent plain agar was 273μ, the maximum was 1,603μ and the average was 824μ. In the first 10 of these tests,

the average growth secured on the best medium in each test was 49 per cent greater than the growth secured on plain agar; in the last 10 tests, the average increase was 216 per cent, which indicates that in spite of the great variability between cultures and the small absolute amount of growth, some progress was made in the development of better agar media (p. 625).

Effect of Various Media on Growth.—In 7 tests in which potato dextrose agar was compared with plain agar, the growth on the latter varied

TABLE 5

RATE OF GROWTH OF GERM TUBES OF *Peronospora destructor* ON AGAR

Time after seeding	Test 1, 13° C, 1.7 per cent plain agar		Test 2, 19° C, 15 cc 1.7 per cent plain agar + 0.5 cc potato dextrose broth + 0.05 cc 1 per cent $KMnO_4$ + 0.5 cc of a melted agar plate culture of *Phytophthora citrophthora*	
	Average length of germ tubes*	Rate of growth per hour in previous interval	Average* length of germ tubes	Rate of growth per hour in previous interval
hours	μ	μ	μ	μ
4	72	18	.....	..
6	136	32	.....	..
8	177	20	.....	..
10	292	57	.....	..
16	.....	..	510	32
21	852	51	.....	..
26	916	13	.....	..
27	.....	..	1,073	51
39	.....	..	1,198	10
41	1,210	20	.....	..
51	.....	..	1,350	13
63	.....	..	1,620	22

* Ten measurements averaged for each value given.

from 546 to 1,603μ with an average of 1,040μ, and on potato dextrose agar the growth varied from 0 to 555μ with an average of 291μ, which indicates that nutrients in potato dextrose agar were reducing growth. The addition of potassium permanganate increased the growth on nearly all agar media tested, and the increase was usually proportional to the content of organic matter. This is illustrated by the results of one test presented in table 6. Here increasing amounts of potato dextrose broth progressively decreased the amount of growth, and the addition of potassium permanganate was progressively more stimulatory as the amount of potato dextrose was increased. The most striking example of this was when potassium permanganate was added to cultures containing an extract from *Rhizopus* cultures. In 1 test, 0.1 cc of a melted, 4-day old *Rhizopus* culture added to 15 cc of plain agar totally inhibited the

growth of *Peronospora destructor,* but the addition of 0.1 cc of 1 per cent potassium permanganate to the plain agar plus *Rhizopus* extract resulted in an average of 1,935μ for the germ tubes. Potassium permanganate was the most consistently stimulatory substance used in these tests.

Other materials which were stimulatory when added to plain agar plus potassium permanganate in numerous tests were glycine, potato dextrose broth, asparagine, riboflavin, potassium nitrate, dibasic sodium phosphate, and melted-agar cultures of *Phytophthora citrophthora.* Sev-

TABLE 6

RELATION OF POTATO DEXTROSE AND POTASSIUM PERMANGANATE TO GROWTH OF GERM TUBES OF *Peronospora destructor*

Amount of 1 per cent $KMnO_4$ added to plain agar	Average length of germ tubes with various amounts of potato dextrose broth added to 15 cc plain agar			
	With 0.0 cc added	With 1.0 cc added	With 2.0 cc added	With 8.0 cc added
cc	μ	μ	μ	μ
0.0	864	899	581	264
0.1	986	1,085	1,008	855
0.2	...	.....	*924	900

eral mixtures were superior to the addition of any single material. The mixtures that were most stimulatory when added to 15 cc of plain agar plus 0.1 cc of 1 per cent potassium permanganate were: (*a*) 1 cc potato dextrose broth plus 1 cc 10 per cent glycine; (*b*) 1 cc potato dextrose broth plus 0.2 cc of 1 per cent potassium nitrate; and (*c*) 2 cc potato dextrose broth plus 1 cc 10 per cent glycine plus 3 cc 10 per cent sucrose plus 0.1 cc 10 per cent dibasic sodium phosphate.

In the foregoing attempts to culture *Peronospora destructor* the possible toxicity of the agar itself was not considered. In 2 tests, germ tubes on 1.7 per cent agar averaged 326μ, on 0.5 per cent agar averaged 633μ, and on 0.2 per cent agar averaged 842μ. Even this value, however, for 0.2 per cent agar is lower than that secured on 1.7 per cent agar in many other tests. Probably there are important differences between different lots of agar, but in 1 test where 2 lots of agar were compared, no marked difference was apparent, and the matter was not investigated further.

An attempt was made to continue the studies on liquid media, but some of the substances, including potassium permanganate, which were stimulatory on agar media, were highly toxic at the same concentration in water.

Some of the instances of apparent stimulation reported may be due to the effect of the test substances in reducing the toxicity of the agar.

In 3 experiments, however, the average and maximum growths in water cultures were less than in agar cultures. Liquid cultures are more difficult to use in such a study for several other reasons, such as the rapid spread of contaminants, and the difficulties of measurement of the germ tubes.

Effect of Host Extracts.—Strips of onion leaves with the internal surface exposed but otherwise uninjured were laid on the surface of seeded plates of plain agar. The spores under and immediately adjacent to the strips did not germinate, but those a few millimeters away germinated normally. Cold-water extracts of onion leaves prepared by grinding onion leaves in 5 times their weight of water and pressing the extract through gauze were poured over the surface of agar and the surplus poured off. Spores seeded on these plates did not germinate. Excised onion leaves were placed on the surface of plain agar and heated for 10 minutes at 90° C and the leaves removed. Spores seeded on this agar surface did not germinate. Apparently some substances are present in the onion-leaf extracts in concentrations toxic to the onion-mildew organism.

The explanation of this apparent anomaly is probably that under natural conditions the parasite does not come in contact with these materials. A variety of more dilute host extracts, however, were apparently stimulatory. In 1 test, 1 part of uncrushed leaves in 20 parts of water was heated for 2 minutes at 100° C and various amounts of the extract added to plain agar. Another extract was prepared by grinding 1 part of leaves in 20 parts of water and using the supernatant liquid after centrifuging. In this test, the germ tubes in the plain agar check averaged 740μ, those cultures with 0.1 cc, 1.0 cc, and 5.0 cc of the extract from crushed leaves averaged 1,004μ, 678μ, and 0μ, respectively.

Effect of Rate of Seeding.—In 1 test, sporangia seeded on plain agar at 0.31 sporangia per sq. mm, 2.3 per sq. mm, and 10.0 per sq. mm, and incubated 48 hours at 13° C gave average germ-tube lengths of 616μ, 974μ, and 1,069μ, respectively, which indicate that heavy seeding favors growth of the germ tubes. The germ tubes from well-isolated single spores grew fairly well, however, and in practice the use of rather light seedings was found desirable for reducing contamination and for securing well-isolated germ tubes that could be easily measured.

Effect of Time of Day and Season.—Seeding of agar plates was generally performed at about 11 a.m. with sporangia produced during the previous night, but sporangia were transferred from sporulating plants to agar at various times of the day and night, and tests were conducted during all months of the year. No diurnal or seasonal variations in the amount of growth from sporangia was apparent. Sporangia 3 days old,

however, showed a lower percentage of germination and a smaller length of germ tubes than those used the morning after sporulation.

Effect of Temperature, Humidity, Light, Oxygen, Carbon Dioxide.—Most of the culture tests were conducted at 19° C, at which temperature the best growth occurred in 1 temperature test. In 2 tests, however, there was good growth with little difference in amount from 10° C (no lower tested) to 19° C with a marked reduction at 22° C and only a trace at 25° C. Exposure of the agar cultures over increasing concentrations of sulfuric acid to reduce the relative humidity decreased the amount of growth in 1 test. Exposure to natural and artificial light reduced the amount of growth slightly in 1 test. In 2 tests, exposure of the cultures over 10 per cent pyrogallol in 45 per cent potassium hydroxide, or over charcoal had no apparent effect on growth.

Adaptation to Agar.—In a series of tests it was attempted to determine if *Peronospora destructor* could be adapted to agar. The idea was that those sporangia which grew best and remained viable for the longest period would be reproduced, and possibly a strain established which was adapted to agar culture. Blocks of seeded agar from plates of test media were used to inoculate onion leaves after specified periods of growth on the agar. Sporangia produced on the onion leaves which were infected by this process were used to seed agar media again. After four generations of this process the selected and original onion-mildew cultures were compared, on the same test media, and no difference in the growth on agar was apparent.

OTHER FUNGI ACCOMPANYING DOWNY MILDEW ON ONIONS

Botrytis cinerea, B. allii, and *Macrosporium* sp. have been frequently observed on plants used in these studies. *B. cinerea* was found fruiting on dead onion leaves in the field and on dead leaves of greenhouse plants that had been incubated overnight in moist chambers. In greenhouse studies, mildew-inoculated leaves with dead tips have frequently given rise to sporulation of *Peronospora destructor* on the living portion of the leaf and to *B. cinerea*[6] on the dead portion. Inoculation with a spore suspension of *Botrytis* from these infected leaves gave rise to elongate, depressed white spots on onion leaves in the greenhouse in 3 days. Such lesions showed no sporulation when the infected plants were incubated in a moist chamber, but after several days such leaves frequently died, and on the dead leaves an abundant sporulation of *Botrytis* occurred in moist chambers. In field plantings a similar white spotting has been observed on onion leaves, and on occasions this spot-

[6] The fungus *Botrytis cinerea* was grown in culture and identified by Dr. H. N. Hansen.

ting (fig. 4) and the killing of the leaves following it, has caused losses in onions grown for greens at Bay Farm Island and San Pablo.

Botrytis allii[7] has not been observed causing localized injury to leaves but is sometimes destructive to seedstalks and entire plants. The characteristic symptoms and signs are a paling of the seedstalks, followed by the formation of concentric arcs or rings of sporodochia or sclerotia. Infection apparently usually starts at the base of the seedstalk and extends upward, but the apparent starting in the flower head and working downward is not uncommon. Seedstalks infected with *B. allii* usually produce very little seed, and in this sense it is more destructive

TABLE 7

DISTRIBUTION OF *Macrosporium* LESIONS ON AUSTRALIAN BROWN ONION SEEDSTALKS, MILPITAS, AUGUST 12, 1937

Treatment	Seedstalks showing no *Macrosporium*	Seedstalks showing *Macrosporium*		
		On all sides	On south side only	On north side only
	number	*number*	*number*	*number*
Control, no treatment (2 plots)	9	163	0	106
Sprayed April 24, 30, May 7, 14, 21, 28 with 2 per cent rosin lime-sulfur (2 plots)	325	3	1	58

than *Peronospora destructor,* for a seedstalk infected with the latter may sometimes have several downy-mildew lesions and yet produce a fair yield of seed. *B. allii* was serious in experimental plantings at Berkeley in 1937 and 1938, and has been observed at Milpitas, San Pablo, and Cotati, but not in the large plantings in the Sacramento–San-Joaquin Delta region or in the southern portion of the Santa Clara Valley. A brief report of *B. allii* and *B. cinerea* on onions has already appeared. (*81*).

Infection with *Macrosporium* has followed that with downy mildew on onion leaves and seedstalks in the field each year from 1935 to 1940, though I have not observed *Macrosporium* on leaves or seedstalks not already infected with downy mildew. Spores of great variability were found on these lesions. Results of one set of observations, presented in table 7, show that spraying with 2 per cent rosin lime-sulfur gave better control of *Macrosporium* than of downy mildew (see also tables 27, 28) and that *Macrosporium* lesions occurred in greatest abundance on the north side of the seedstalks. This is possibly because infection with *Macrosporium* requires a long period with moisture on the seedstalks, and the rain or dewdrops remained for a longer period on the north side.

[7] The fungus *Botrytis allii* was also cultured and identified by Dr. H. N. Hansen.

CARRYOVER OF ONION MILDEW FROM ONE SEASON TO ANOTHER

The manner in which onion downy mildew lies dormant during periods of inactivity of the host, and by means of which it is carried over from one crop to a succeeding one has been subjected to considerable speculation and experimental study. Because of differences in climate and manner of onion culture, this seasonal perpetuation consists in overwintering in some regions of cold winters as in New York, of oversummering in some regions of mild winters and dry, warm summers as in California, and of overwintering and oversummering as reported by Katterfeld (*31*) in Russia. The methods of carryover which should be considered are: (*a*) carryover as mycelium in the bulbs, seed, or soil, (*b*) carryover as oöspores on or in seeds, or in the soil or refuse, (*c*) seasonal reintroduction by wind or other agencies from an infected region to a noninfected crop.

CARRYOVER AS MYCELIUM

Carryover of onion downy mildew as mycelium in dormant bulbs was suggested by Trelease (*69*) and Dudley (*16*), but was first established by Murphy (*43*) in Ireland in 1921.

On march 15, 1935, at Cotati, 1 plant with small, pale, down-curled, heavily sporulating leaves was observed in a field of late-planted onions showing considerable secondary downy-mildew infection. A total of 1,000 plants was then examined but no more such plants were found. The stunted development of this plant indicates that it probably grew from a systemically infected mother bulb. The possibility that it became infected after planting in the field is not excluded, but is unlikely since no other similarly infected plants could be found.

On February 25, 1935, a group of bulbs from a heavily infected 1934 crop of bulbs grown at Davis were planted in the greenhouse at Berkeley. Of 4 of the Sweet Spanish and 9 of Australian Brown which grew by March 17, only 1 of the Sweet Spanish plants showed symptoms of infection, and this plant produced an abundance of sporophores on overnight incubation in a moist chamber.

On March 25, 1937, most of one day was spent in carefully examining random areas in several hundred acres of onions of numerous varieties grown for seed in various locations in the Santa Clara Valley. In none of these fields was any secondary mildew infection found, and only 1 systemically infected plant—and this without sporulation—was found.

On August 25, 1938, bulbs of 3 strains of Yellow Bermuda, 1 of Red Creole, and 1 of Early Grano, all from a heavily infected bulb crop

grown at Milpitas in 1937–38, were planted in Berkeley outdoors. These varieties were chosen because they were among the most severely infected in a block of several varieties, the leaves of which were killed by mildew. On October 4, the plants grown from this heavily infected bulb crop showed no secondary mildew infection and only 5 systemically infected plants; 3 of the latter are illustrated in figure 2.

On October 19, 1938, other lots of plants grown from bulbs from these heavily infected 1937–38 Milpitas fields were examined at Milpitas. Seven systemically infected plants were found among 1,674 Stockton Yellow Globe plants, but no systemic infection was found in 226 Lord Howe Island plants or in 244 plants of Early Grano. The scarcity of systemic infection in bulbs from plants known to be heavily infected, emphasizes the relatively small amount of carryover as systemically infected bulbs in California.

In addition to the detailed observations already given, many thousands of onion plants grown for seed in 1935–1940 were examined for evidence of carryover of the disease in dormant bulbs and none were found, though many of the mother bulbs were known to come from crops showing mild to severe mildew infection. If my observations and conclusions are correct, the situation in California is in marked contrast to the condition in Ireland (*44*) where as high as 100 per cent infection has been found in dormant bulbs.

While systemically infected bulbs apparently responsible for the oversummering of onion mildew in California are rare, systemically infected field plants are less rare. On May 24, 1935, all plants of a block of Red Wethersfield grown for bulbs on Liberty Island were systemically infected, but no other variety showed severe systemic or secondary infection. Twenty of the Red Wethersfield plants were dug up and potted in Berkeley. Only 2 survived; these were weak and no sporulation could be induced on them. On January 12, 1938, a plot of Yellow Bermuda grown for seed at Milpitas was examined and no systemic infection was noted, though there was considerable local infection on the leaves. On March 21, 104 out of a total of 717 of these same plants showed systemic infection. I believe that this systemic infection occurred after the bulbs were planted and did not originate from dormant mycelium in the bulbs. On March 14, 1939, a field of plants grown for bulbs at Salinas showed 36 plants with systemic infection out of 100 counted.

In the early stages of this study, tissues from several bulbs from crops known to be heavily infested with mildew were examined microscopically but no downy-mildew mycelium was found. This is not surprising in view of the scarcity of naturally occurring systemically infected plants resulting from an infected bulb crop. In plants showing

systemic infection, however, downy-mildew mycelium could be found continuous from the leaves to the growing plate at the base of the bulb in all cases. While detection of the fungus in the dormant bulbs as was done by Murphy and McKay (*44*) is desirable evidence that they are actually infected, I consider the production of systemically infected plants from dormant bulbs under conditions that preclude infection from other sources to be good evidence that the bulbs were sytemically infected and could serve to carry over the disease. Mildew mycelium has been found in dormant bulbs several days after artificially inoculating them with a spore suspension, but in only small amounts.

An attempt was made to determine the factors underlying the occurrence of systemic infections, but without success. Systemic infection occasionally developed on plants grown in the greenhouse from healthy bulbs inoculated by spraying the healthy leaves with a spore suspension, but the number of plants systemically infected under these conditions seemed too low to use this method for further study. On seedling plants, however, a high percentage of systemic infection frequently developed, and inoculated seedling plants were subjected to various light, temperature, and leaf-pruning treatments without apparently significant differences in the incidence of systemic infection. Since systemic infection appeared to occur more frequently in the winter months in field and greenhouse plantings, however, I believe that slow rate of growth of the onions or low temperatures are favorable for the occurrence of systemic infection.

In the field of Stockton Yellow Globe at Milpitas in 1938–39, all plants showing systemic infection were staked on November 7, 1938. On February 20, 1939, at which time there was considerable sporulation and secondary infection, a few systemically infected plants were found in addition to those already staked. As these unstaked, systemically infected plants were larger than the staked plants and had shown no symptoms on November 7, I believe that these plants were infected after November 7. On February 20, some of the sytemically infected plants staked on November 7 were dead, and the bulbs decayed. The comparative appearance on February 21 of plants without systemic infection, those which showed systemic infection on November 7, and those on which systemic infection showed up later is illustrated in figure 1.

SEED TRANSMISSION NOT DEMONSTRATED

Onion downy mildew might be carried over in the seed as viable mycelium or oöspores in the seed or as oöspores on the seed. Katterfeld (*31*) found mycelium and oöspores in the pedicels of the flowers and almost reaching the base of the ovary but could not find mycelium in

the seed. He believed that mycelium could not enter the pedicels if infection occurred after the formation of the seedstalks. Hiura (*25*) found mycelium in the flower stalks, perianths, styles, ovaries, filaments, and anthers, but not in the ovules. Cook (*12*) found mycelium in the ovules. Chapman (*9*) found onion-mildew spores, presumably oöspores, in 10 out of 10 lots of onion seed. Cook found a few oöspores in several lots of onion seed. Katterfeld (*31*), Murphy and McKay (*44*), and Hiura (*25*), however, obtained only healthy plants from seed from mildew-infected plants. Field observations have been used as evidence of seed transmission. Newhall (*47*) states, "There is also evidence in many fields that seed was in several cases responsible for initial infection this year as blight appeared as early and spread as rapidly on muck growing its first crop of onions as on old onion soil." Later Newhall (*49*) made extensive tests of seed treatment as a means of onion-mildew control, but reported no apparent success.

To determine the possibility of seed infection in California, seed from the variety Prizetaker was saved in 1935 from 12 seedstalks with mildew infection just below the head. Most of the seed was shriveled, but 440 seeds grew and no mildewed seedlings resulted from this seed. Bulk seed from the same heavily mildewed crop also showed no infection in the resulting seedlings. In another test, green healthy seed heads on field plants were inoculated on August 6, 1935, by spraying them with a spore suspension and incubating them overnight in moist chambers. From these inoculated heads, 343 seeds grew and none of the plants showed infection. I feel that there is no good evidence that onion mildew may be carried over from one season to another with the seed.

Spread by Oöspores.—Jones (*30*) scattered oöspore-bearing refuse from mildewed onions on a greenhouse plot and planted this and a control plot with onion seed in December. In April, mildew appeared in the plot in which refuse had been placed but not in the control plot. Murphy and McKay failed to get infection from refuse from mildewed onions in tests reported in 1926 (*45*) but in 1932 they reported that naturally contaminated soil freely conveyed the disease to seedling onions.

Germination of an oöspore of onion mildew was first reported by Murphy and McKay (*46*). They reported that a 6-month-old oöspore which had wintered in the laboratory produced a slender hyphal thread bearing about 24 conidia on short, irregularly arranged branches. In later, more extensive tests, McKay (*36*) reported that germination of oöspores occurred by means of germ tubes, and only with oöspores four years or more in age. In spite of the small amount of evidence, oöspores must be recognized as a potential source of primary infection of onion downy mildew.

Spread by Sporangia.—Doran (*14*) has given circumstantial evidence that infection of cantaloupe downy mildew in the northeastern United States may arise from air-borne sporangia produced on plants farther south. A similar situation is possible with onion downy mildew. In the San Francisco Bay region, specifically in the Colma and Bay Farm Island districts, onions are grown for greens throughout the year, and downy mildew has been found in these districts at all seasons. As the principal seed-producing areas are within 100 miles of this region, as the prevailing winds are principally west, southwest, and northwest from the Bay region (*5*), and as sporangia are viable for several days, the opportunity for sporangia from the Bay region to cause infection of onions grown at Cotati, in the Sacramento–San-Joaquin Delta region, and in the Santa Clara Valley appears good.

Of the four principal methods by which onion mildew might originate, namely, (1) systemic mycelium in the bulbs, (2) oöspores in soil or refuse, (3) sporangia from regions where onion mildew prevails throughout the year, and (4) infected seed, I believe the first is of greatest importance in California, the second two are of minor importance, and the fourth is of very little importance or nonexistent.

FACTORS AFFECTING FORMATION OF SPORANGIA

Sporangiophores and sporangia of onion downy mildew are normally formed on leaves and seedstalks under conditions of high humidity at night. Conditions governing sporulation might be divided into those preceding and those during sporulation. According to my conception of the diurnal cycle of sporulation (*79*), the host tissues must build up during the light portion of the day a reserve of labile materials necessary for sporulation. Although under natural conditions light is probably never a limiting factor in this process, nevertheless, under controlled conditions infected greenhouse plants sporulated best when transferred to dark, moist chambers at 4 p.m. to 10 p.m. and sporulated poorly or not at all when transferred to dark, moist chambers at 12 p.m. to 1 a.m. Also plants held in a dry, dark environment for 24, 36, or 48 hours could not be induced to sporulate by placing them in dark, moist chambers at 13° C, but sporulation was made possible by exposing them to light for a few hours. These facts are in accordance with the theory that food reserves are necessary to sporulation. Attempts to show that the labile product necessary for sporulation was sucrose were not successful. Infected leaves that had been held in the dark were placed with their bases in 5 per cent sucrose or floated on 10 per cent sucrose in the dark for various times, but these treatments did not induce sporulation. Also excised leaves from plants that had been held in

the dark were exposed to light in sealed chambers containing an excess of potassium hydroxide to remove carbon dioxide and reduce photosynthesis, and these leaves showed sporulation after being placed in dark, moist chambers.

No data under controlled conditions of the effect of temperature during the light portion of the day on the subsequent sporulation at night have been secured, but I believe that high temperatures during the day are sometimes responsible for poor sporulation the following night. In the first clear warm days of spring, before the greenhouses were whitewashed to reduce the temperature, it was not uncommon for the greenhouse air temperatures to go up to 35° and 48° C for short periods and to fall to 12° to 18° C at night. On several such occasions mildewed onions have failed to sporulate in moist chambers at night; this was no doubt due to the high temperature during the light portion of the day, which is probably also sometimes responsible for failure of sporulation under field conditions. With greenhouse day temperatures probably never going below 18° C, low day temperature has apparently never been responsible for sporulation failures, but under field conditions in winter when sporulation is very erratic, low day temperatures might be responsible for the failure of subsequent sporulation in some cases.

Humidity, temperature, and light are the principal factors acting during sporulation to determine its success. Under greenhouse conditions, with the observed relative humidity at night varying from 65 to 81 per cent, onion mildew rarely sporulated, though luxuriant sporulation occurred at similar temperatures when the plants were incubated overnight in moist chambers. Low humidity is apparently also frequently responsible for the failure of infected plants to sporulate under field conditions. To determine more accurately the effect of humidity on sporulation, onion leaf tissues with their bases in cotton-stoppered vials of water were placed in sealed chambers containing water or dilute sulfuric acid to produce specified relative humidities (*63*). Sporulation was rated on an arbitrary relative scale of 0 to 10, in which 0 indicated no sporulation and 10 indicated luxuriant sporulation. Results of 3 tests at 16° C were as follows:

Relative humidity, in per cent	December 18, 1935, sporulation rating	December 20, 1935, sporulation rating	January 7, 1936, sporulation rating
100	10	10	10
97	10	5	5
94	10	1	0
90	5	0	0
80	0	0	0

These results indicate an optimum of about 100 per cent relative humidity for sporulation, with a minimum of about 90 per cent.

Humidity is apparently an important factor in determining the size of the sporangiophores, though no data were collected from controlled tests. On the morning of March 29, 1937, some plants exposed on the greenhouse bench showed considerable sporulation, though not so much as similar plants incubated overnight in a moist chamber at the same temperature. From mounts of the sporangiophores produced on each

TABLE 8

EFFECT OF TEMPERATURE ON SPORULATION* OF ONION DOWNY MILDEW

Temperature	Excised leaves		Potted plants		Average
	5 p.m. December 18, 1935	8 p.m. July 6, 1937	5 p.m. February 10, 1939	6 p.m. February 23, 1939	
°C	*rating*	*rating*	*rating*	*rating*	*rating*
4	0	0.0	...	0.0	0.0
7	0	0.0	2.0	5.7	1.9
10	8	5.1	4.9	6.0	5.7
13	10	...	9.0	7.1	8.7
16	5	7.9	8.0	6.7	6.9
19	6	0.2	1.3	0.2	1.9
22	3	0.1	1.1	3.7	1.9
25	0	0.0	0.0	0.0	0.0
28	0	...	...	...	0.0

* Scale of 0 (no sporulation) to 10 (luxuriant sporulation).

set of plants, the length of 10 random sporangiophores was determined. Those produced in the open greenhouse ranged from 122μ to 167μ and averaged 150μ; those from the moist chamber ranged from 317μ to 828μ and averaged 521μ.

To determine the effect of temperature on sporulation, potted plants or pieces of onion leaves were transferred to moist chambers and held at various temperatures in the dark. Results of 2 tests with excised leaves and 2 with potted plants are given in table 8. The results indicate a minimum temperature for sporulation of 4° to 7° C, an optimum of about 13° C and a maximum of 22° to 25° C. The temperature range for sporulation is therefore lower than that for germination and infection.

Temperatures prevailing during sporulation affect the size of the sporangiophores. In 2 tests, summarized in table 9, the sporangiophores averaged 324μ at 7° C, 593μ at 16° C, and 480μ at 22° C. From the data of table 9 there is no apparent consistent effect of temperature on size of sporangia, but these data are probably inadequate.

Sporulation of onion mildew in daylight has not been observed to occur, and infected greenhouse plants placed at 8 to 10 a.m. in moist

chambers exposed to daylight of cloudy days have failed to show sporulation by 5 p.m. on several occasions. In 3 out of 6 tests, however, sporulation occurred in artificial light at night. In these tests excised leaves were placed at 21° C in a sealed glass moist chamber immersed in a water bath and exposed at a distance of 6 cm or more to a 150-watt Mazda lamp giving from 160 to 1,000 foot-candles of light on the leaf surface. Control leaves were placed in darkness at 22° C, a temperature far above the optimum for sporulation but under which conditions fairly satisfactory sporulation occurred in these tests. In 5 of the 6 tests, sporulation occurred on the control leaves and in 4 of the 5 successful

TABLE 9

EFFECT OF TEMPERATURE ON SIZE* OF SPORANGIOPHORES AND SPORANGIA

Temperature	Test of February 11, 1939			Test of February 24, 1939	
	Length of sporangiophores	Length of sporangia	Diameter of sporangia	Length of sporangiophores	Length of sporangia
°C	μ	μ	μ	μ	μ
4	0	0	0	0	0
7	264	65	27	384	60
10	299	58	26	448	63
13	459	54	23	600	58
16	554	63	24	632	55
19	320	51	22	431	51
22	412	56	21	548	64
25	0	0	0	0	0

* Each value is the average of 10 measurements.

tests the sporulation was slightly more luxuriant in darkness than in light, but in 1 test the sporulation was apparently more successful in light. Sporangia formed under these conditions of artificial light at night were abnormal in shape in that they were frequently constricted in the center. From these tests of the effect of light before and during sporulation, it appears that exposure of infected plants to light favors subsequent sporulation in darkness, that during sporulation light is unfavorable to the sporulation process, and that the normal diurnal cycle of sporulation of onion mildew in nature is in part an adaptation to the alternation of light and darkness in the normal day.

In 2 tests the epidermis was peeled from portions of infected leaves, and the leaves were opened to expose their inner surfaces, which normally face the inner cavity, before placing them in moist chambers. In both tests sporulation occurred on the surface from which the epidermis was removed, but it was estimated that only about one tenth as many sporangiophores were formed on this surface as on the normal surface. Sporulation did not occur on exposed or unexposed inner surfaces of the onion leaves.

MATURATION AND DISSEMINATION

At 8 p.m., April 14, 1936, infected plants were transferred from the dry greenhouse bench to moist chambers at 18° C. At 12 p.m. infected leaves showed an abundance of sporangiophore initials with occasional branching but with no sporangia. At 3 a.m., April 15, the sporangiophores were completely developed and the sporangia were about one third their mature size. At 6 a.m. the plants were returned to the dry greenhouse bench. The sporangia appeared mature, but were not readily released. At 7 a.m. the sporangia were readily released by shaking the leaves. The mechanics of sporangium release was not studied, but generally observations indicate that it is a passive phenomenon which depends on agitation of the leaves and on air currents. When sporulating leaves are removed from a moist chamber to a dry environment a slow turning of the sporangiophores, less pronounced than with hop downy mildew, is apparent, but this movement does not appear very effective in discharging the sporangia.

The liberation of sporangia from sporulating plants was followed by periodically changed spore-trap slides in 3 tests. At 8 a.m., December 12, 1935, a heavily mildewed plant with a fresh first crop of sporangiophores was tranferred from a moist chamber to the dry greenhouse bench and one slide was placed on the bench on each of two opposite sides of the plant. In the interval from 8 a.m. to 10 a.m., 2 sporangia were found on 3.2 sq. cm of area on the slides; from 10 a.m. to 12 m., 30 sporangia; from 12 m. to 3 p.m., 166 sporangia; and from 3 p.m. to 6 p.m., 71 sporangia. The temperature and relative humidity were 14° C and 71 per cent, respectively, at 10 a.m. and 18° C, and 48 per cent at 3 p.m. From these results it would appear that maximum liberation of sporangia occurred between 12 m. and 3 p.m.

In another test, started at 7 p.m., June 14, 1936, 3 plants were placed in a dark moist chamber at 13° C and 6 in a light greenhouse moist chamber and slides were placed beside the plants. At 6 a.m., June 15, the plants were sporulating luxuriantly, and the sporangia appeared mature but none were collected on the slides. Three of the plants from the light greenhouse moist chamber were transferred to the dry greenhouse bench. At 8 a.m. a total of 37 sporangia were counted on 0.4 sq. cm on each of 3 slides beside plants in the dark moist chamber at 13° C, none on slides beside the plants in the greenhouse moist chamber, and 2 sporangia on the slides beside the plants on the greenhouse bench. At 2 p.m., 290 sporangia were counted on 1.2 sq. cm of slides beside the plants in the 13° C dark moist chamber, 10 on the slides beside the plants in the greenhouse moist chamber, and 377 on the slides on the greenhouse

bench. The high number of sporangia caught from plants in a dark moist chamber was unexpected, but the test was not repeated.

At 10:20 a.m., February 19, 1941, 9 spore-trap slides were exposed on the ground in various positions on all sides of a plot of onions showing fresh sporulation of onion mildew at Milpitas. At the end of each test

TABLE 10

Longevity of Onion-Mildew Sporangia Attached to Sporangiophores on Original Sporulating Leaves

Time of start of test*	Condition of exposure†	Period of exposure	Germination
		hours	*per cent*
11 a.m. January 24, 1936	Detached leaves,‡ greenhouse	81	58
9 a.m. January 25, 1936	Detached leaves,‡ greenhouse	56	91
8 a.m. March 14, 1936§	Normal	12	50
9 a.m. April 9, 1936	Normal	46	98
9 a.m. April 9, 1936	Normal	57	34
9 a.m. April 9, 1936	Detached leaves,‡ outdoors	46	13
9 a.m. April 9, 1936	Detached leaves,‡ outdoors	54	0
8 a.m. April 17, 1936	Normal	72	40
8 a.m. April 17, 1936	Normal	96	2
8 a.m. April 17, 1936	Normal	120	0
8 a.m. May 6, 1936	Greenhouse	5	50
8 a.m. May 6, 1936	Greenhouse	192	0
8 a.m. April 2, 1937	Normal	72	0
8 a.m. April 2, 1937	Greenhouse	72	86
8 a.m. April 2, 1937	Greenhouse	96	28
8 a.m. April 2, 1937	Greenhouse	144	18
8 a.m. April 2, 1937	Detached leaves,‡ greenhouse	72	55
8 a.m. April 2, 1937	Detached leaves,‡ greenhouse	96	0
9 a.m. April 6, 1937	Normal	24	83
9 a.m. April 6, 1937	Detached leaves,‡ outdoors	24	1

* Time of removal from moist chamber of freshly sporulating plants which were later used as a source of sporangia.

† On turgid living leaves attached to potted plants outdoors unless otherwise mentioned.

‡ Leaves cut from sporulating plants and exposed in dry open petri dishes. Such leaves usually wilted in a few hours and became brittle in a few days.

§ Naturally sporulating field plants. The 8 a.m. time and 12-hour exposure period are approximations.

interval 0.4 sq. cm of each slide was examined for sporangia and a clean slide was exposed in the same position. The number of sporangia caught at the different positions varied with the prevailing wind and with the proximity to sporulating plants. For all slides and all positions, the number of onion-mildew sporangia per hour per sq. cm of slide was 57 from 10:20 to 10:45 a.m., 22 from 10:45 to 11:20 a.m.; 15 from 11:20 a.m. to 12:20 p.m.; 4.5 from 12:20 to 1:40 p.m.; 4.1 from 1:40 to 2:40 p.m.; and 3.2 from 2:40 to 3:35 p.m.

On the basis of these 3 limited tests, I believe that the maximum liberation of sporangia of onion mildew is a few hours after the sporangia are apparently morphologically mature and that the rate falls off progressively from around midday to the next maturation of spo-

rangia. Under greenhouse and field conditions, however, sporangiophores and many sporangia may remain attached to the leaves for several days.

LONGEVITY OF SPORANGIA

The longevity of sporangia of onion mildew was meausred in 16 tests, from which sample data are presented in tables 10 and 11. These tests were designed to simulate natural conditions, and to measure the longevity of sporangia when attached to the sporangiophores on turgid leaves,

TABLE 11

LONGEVITY OF ONION-MILDEW SPORANGIA ON DRY LEAVES OF HEALTHY PLANTS

Time of start of test*	Age of sporangia†	Condition of exposure of healthy plants seeded with sporangia	Period of exposure	Plants inoculated	Plants infected
	hours		*hours*	*number*	*per cent*
9 a.m. April 9, 1936	1	Outdoors	10	10	40
9 a.m. April 9, 1936	58	Outdoors	0	23	55
9 a.m. April 9, 1936	58	Outdoors	4	16	6
8 a.m. September 23, 1936	1	Greenhouse	0	15	73
8 a.m. September 23, 1936	1	Greenhouse	24	19	10
8 a.m. September 23, 1936	1	Greenhouse	72	19	0
8 a.m. September 23, 1936	1	Outdoors	72	15	13
8 a.m. April 27, 1937	8	Outdoors	3	66	42
8 a.m. April 27, 1937	8	Outdoors	28	117	0
8 a.m. May 25, 1937	13	Outdoors, sun	24	68	0
8 a.m. May 25, 1937	13	Outdoors, shade	24	71	31
8 a.m. May 25, 1937	13	Greenhouse, sun	24	64	41

* Time of removal from moist chamber of freshly sporulating plants which were later used as a source of sporangia.

† Age of sporangia is given as from the time when the sporulating plants were removed from the incubator to the time when the sporangia were dusted onto healthy plants.

when attached to the sporangiophores on dead leaves, and when detached and lying on the surface of healthy leaves. To accelerate the drying out and death of leaves, the test leaves bearing sporangiophores and sporangia were cut from plants and exposed in open petri dishes to the test environment. Germination tests were performed by seeding the test sporangia onto plates of plain agar, and after an adequate incubation period 100 sporangia were counted for each germination percentage recorded. To determine the percentage infection, the healthy plants seeded with sporangia were sprayed with water, placed overnight in a moist chamber, left on the greenhouse bench for 12 days, incubated in a moist chamber, and the number of plants showing sporulation was counted. In all but 2 of the tests, control sporangia taken at the time of removing the sporulating plants from the moist chambers germinated 90 per cent or above, and the low percentages in tables 10 and 11 must be attributed to the injury caused by the subsequent environment. With sporangia dusted onto plants, however, results were rather erratic, and

100 per cent infection was rarely secured. All tests were performed in the absence of rain but with no other selection of weather conditions. From the results of tables 10 and 11, it appears that the sporangia of onion mildew may live for 3 to 5 days on the sporangiophores of turgid leaves outdoors, but for a lesser period on detached wilted leaves, and as detached sporangia on healthy leaves in the outdoor environment in Berkeley. Another important epidemiological factor, the length of time the sporangia may remain viable while being disseminated by wind, was not studied, but Newhall (*49*) has reported that sporangia caught in the air up to 1,500 feet elevation were still viable.

TABLE 12

EFFECT OF TEMPERATURE ON GERMINATION OF SPORANGIA OF ONION DOWNY MILDEW

Temperature	Germination in water substrate				Germination on agar substrate	
	4 p.m. Nov. 8, 1935	9 a.m. Nov. 23, 1935	4 p.m. Dec. 4, 1935	4 p.m. Dec. 6, 1935	5 p.m. Feb. 5, 1936	11 a.m. Apr. 27, 1936
° C	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2	0	1	8	0	95
4	4	22	4	11	94	96
7	2	22	5	50	93	98
10	..	19	3	15	99	97
13	3	..	13	19	95	92
16	20	18	16	18	96	94
19	9	20	6	12	96	99
22	..	16	5	15	91	53
25	..	0	6	0	70	0
28	..	0	0	0	0	0

GERMINATION OF SPORANGIA

The sporangium of onion mildew germinates by the formation of a germ tube which usually arises from near the proximal, pointed end of the sporangium, and the germ tube is frequently constricted at the point of origin from the sporangium. To determine if free water was necessary for germination, sporangia were dusted onto slides on which drops of water had been placed, and the slides were incubated in petri-dish moist chambers at 16° C. After 16 hours the sporangia in and on the drops of water showed a high percentage of germination, but those on the dry glass beside the drops showed no germination.

With potted plants, however, as heavy infection was secured when dry plants were dusted with dry sporangia and placed overnight in a moist chamber at 13° C as when similarly inoculated plants were sprayed with water before being placed in the moist chamber (*84*).

The effect of temperature on the germination of sporangia was measured in 6 tests which are summarized in table 12. In the tests of Novem-

ber 8 to December 6, 1935, drops of spore suspension were placed on the slides; in the other 2 tests sporangia were dusted onto plates of plain agar. Germination counts of 100 sporangia per treatment were made about 18 hours after seeding. The results are not consistent but indicate that germination may occur from 1° C (no lower tested) to about 28° C, with no apparent difference in the amount of germination from 7° to 16° C. The high germination in some tests at 1° C is of interest.

TABLE 13

DISTRIBUTION OF DOWNY-MILDEW INFECTION IN ONION FIELDS AS RELATED TO PREVAILING WIND*

Date and location	Plants infected				
	North side	East side	South side	West side	Center
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
April 22, 1935, San Pablo, field 1	51	98	48	2	46
April 22, 1935, San Pablo, field 2	..	100	..	0	..
April 23, 1935, Modesto	..	100	..	94	..
April 24, 1935, Cotati, field 1	24	88	78	62	..
April 24, 1935, Cotati, field 2	99	88	..	62	..
June 3, 1936, Cotati	27	..	3	..	..
April 22, 1938, Walnut Grove	10	38	25	7	36
April 29, 1938, West Sacramento	72	78	56	4	87
July 13, 1938, Cotati, field 1	2	1	14	3	..
July 13, 1938, Cotati, field 2	11	..	13	6	..
Bay Farm Island	..	76	..	0	..
Average	36	74	34	24	..

* Prevailing winds at these locations from west and south.

The effect of light on germination was measured in only 1 test. One dish of sporangia was exposed at about 13° C to about 100 foot-candles of light from a Mazda lamp and a similar mount was exposed to the dark. After 18 hours, germination in the light was 87 per cent and in the dark was 98 per cent. The germ tubes formed in the light were smaller in diameter and shorter than those formed in darkness.

SECONDARY CYCLES

Secondary cycles of onion mildew arise from sporangia disseminated through the air from plants with primary infection—usually systemic infection. I have never observed systemic infection to be the direct cause of serious losses in California; the widespread crop damage is caused by secondary infection. Systemically infected plants from bulbs planted for a seed crop have shown sporulation in October; and since sporangia may produce lesions ready to sporulate in about 10 days, many secondary cycles of the fungus may have occurred before the destructive

epidemics which usually occur in March, April, and May. Wind dissemination of the sporangia of onion downy mildew has been recorded by several investigators but Newhall (*48*) was perhaps the first to record the recovery of onion-mildew sporangia from the air on spore traps. Newhall records catching sporangia in air over onion fields to elevations of 1,500 feet.

The distribution of onion-mildew infection in several California fields afforded good circumstantial evidence of the wind-borne nature of the disease (table 13). In most cases, infection was heavier on the leeward side of onion fields with respect to the prevailing winds. After infection becomes severe, differences in different parts of the field may be no longer apparent. Table 13 records all cases where counts were made, but cases were seen where all plants were infected on all sides of the field.

INOCULATION AND INFECTION

Artificial Inoculation Methods.—In nature, inoculation probably occurs almost exclusively by the transfer of sporangia from a sporulating leaf to a healthy leaf by the agency of wind and minor air currents. In this study the following methods of inoculation have been tested:

1. Dusting dry sporangia onto dry or wet leaves or seedstalks.
2. Spraying a spore suspension onto leaves and seedstalks.
3. Applying a wet cotton swab containing sporangia to seedstalks.
4. Applying agar blocks containing sporangia to leaves and seedstalks.
5. Injection of a spore suspension into the internal cavity of leaves and seedstalks.
6. Injection of a spore suspension into dormant bulbs.
7. Inserting pieces of infected leaf tissues into dormant bulbs.

All methods were successful except the last.

Dry sporangia were used extensively as inoculum in tests on the effect of free moisture on infection with onion mildew and other fungi (*84*), and in inoculating field plots. The method as used in greenhouse cultures was to lay the healthy plants to be inoculated in the bottom of a large can and dust sporangia on them from above.

Spraying with a spore suspension was the most widely used method of inoculation in this study. In 10 consecutive fungicide tests from February 9 to June 12, 1936, the percentage infection of the control seedling plants inoculated by spraying with a spore suspension varied from 62 to 100 per cent, with an average of 88 per cent. Much better control and knowledge of the distribution of the inoculum is possible by using a spore suspension than by using dry sporangia; and an abundance of free moisture, believed desirable for the germination of the sporangia and for maintaining a high humidity, is provided with the inoculum. One

infected sporulating leaf can be used to inoculate hundreds of plants by this method.

Inoculation by applying a wet cotton swab containing sporangia was successfully used in 2 field tests on dry nights when individual seedstalks were to be inoculated without inoculating others. Strips of absorbent cotton about 3 inches long were dipped in a spore suspension and wrapped around the seedstalks.

Agar blocks containing sporangia were used in greenhouse tests where it was desired to inoculate leaves in a specific location, or where it was desired to test the infectivity of cultures growing on test agar media. Sporangia were dusted onto the cooled test agar plates, and small squares were cut from these plates and applied to the surface of onion leaves, to which they usually adhered if applied carefully.

The injection of a spore suspension into leaves and seedstalks was used in field and greenhouse inoculations. By this method it was possible to inoculate individual leaves without contaminating others, and no moist chamber incubation was necessary. The method was successful in greenhouse tests, but gave only a low percentage of infection in 1 field test. In the field many of the seedstalks inoculated in this way became infected with *Botrytis allii*.

Inoculation by injecting a spore suspension into dormant bulbs was also widely used in this study. Bulbs ranging in size from 3 to 100 grams were injected at their centers with a trace to about 1 cc of spore suspension with a hypodermic syringe. The syringe needle was inserted to about the center of the bulb, withdrawn about a millimeter and compressed, which forced a considerable amount of the spore suspension between the leaf bases. The success of this method might be considered surprising in view of the known toxicity of onion sap to the sporangia. It is likely, however, that by this manner of injection, the sporangia were forced beyond the region of injury, to the surface of normal leaf bases where infection started. By this injection method, systemically infected plants indistinguishable in growth symptoms from naturally occurring systemic infection have been produced. Occasionally only 1 or 2 of the growing points from the bulb are infected by this manner of inoculation, but such partial systemic infection also occurs under natural conditions. The inoculated bulbs grow as readily as noninoculated bulbs, and usually produce leaves large enough for sporulation in about 2 weeks under greenhouse conditions. Murphy and McKay (*44*) tried several methods of direct inoculation of bulbs, including inserting sporangia into the bulbs, but secured no infection by any method. The failure may have been because the sporangia remained localized near the wounded tissue.

Penetration Through Stomata.—The germ tubes of *Peronospora destructor* penetrate the onion through the stomata on leaves and seed-stalks. In my observations an appressorium was usually formed by the germ tube over the stoma, and a vesicle was usually formed in the substomatal cavity (fig. 11, *E, F*), but no appressorium is illustrated by Trelease (*69*), Shipley (*60*), Whetzel (*76*), or Katterfeld (*31*). In 1 test on December 13, 1935, 53 stomatal penetrations showed 42 with appressoria apparent. In a test on September 24, 1936, 27 stomatal penetrations were observed in an 11-hour culture at 13° C in dark, moist chambers, and of these 16 showed definite appressoria over the stomata, 4 showed no appressoria, and 7 were doubtful.

Penetration of the stomata by the germ tubes from onion-mildew sporangia causes the nuclei of the ordinary elongate onion-leaf epidermal cells to move toward the penetrated stomata. Results of one set of observations 11 hours after inoculation at 13° C in the dark were as follows:

Condition of nuclei of inoculated leaves	Number
Nonpenetrated stomata (20 observed)	
Nuclei of adjacent cells in approximately central position	20
Nuclei of adjacent cells showing displacement from central position	0
Penetrated stomata (20 observed)	
Nuclei of adjacent cells in approximately central position	1
Nuclei of adjacent cells showing displacement from central position	19
Nuclei of laterally adjacent cells showing migration toward penetrated stomata	18
Migration in 1 cell only	2
Migration in both cells	16
Nuclei of terminally adjacent cells showing migration toward penetrated stomata	6
Migration in 1 cell only	3
Migration in both cells	3

This response of the nuclei adjacent to penetrated stomata is similar to that observed by Caldwell and Stone (*6*) with leaf-rust of wheat.

Effect of Temperature on Infection.—The effect of temperature on infection was determined in 4 tests which are summarized in table 14. In the first 3 tests, dry dormant bulbs were inoculated hypodermically and the inoculated bulbs were incubated at the test temperatures for 48 hours, after which time they were planted in the greenhouse. Infection was determined from the symptoms of systemic infection which were apparent 3 weeks after inoculation. In the hypodermic inoculations there were only 4 bulbs for each temperature treatment in each test, and in some cases only 3 bulbs grew. Infection occurred from 1° to 28° C. In 1 test, seedling plants growing in 4-inch pots were inoculated by

spraying with a suspension of sporangia, and the plants were placed in moist chambers at the test temperature for 16 hours and then placed in the greenhouse. Infection occurred from 4° to 25° C.

Incubation Period about 6 Hours.—Incubation is here used as the development of the fungus from inoculation until it establishes nutritional relations with the host. This was followed microscopically in 1 test and culturally in several tests. In the penetration process the protoplasm of the sporangium tends to mass toward the tip of the germ tube,

TABLE 14

EFFECT OF TEMPERATURE ON INFECTION WITH ONION DOWNY MILDEW

Temperature	Bulbs inoculated hypodermically			Plants inoculated by spraying with a spore suspension, 4 p.m. February 28, 1938
	2 p.m. August 17, 1936	August 25, 1937	4 p.m. September 4, 1937	
° C	*per cent*	*per cent*	*per cent*	*per cent*
1	50	...	...	0
4	75	100	100	100
7	33	...	...	100
10	33	100	100	100
13	100	100	100	100
16	75	100	...	100
19	75	50	...	100
22	0	...	100	100
25	50	...	100	9
28	0	...	100	0

and as growth progresses the sporangium is first emptied of its protoplasm, then the germ tube, and then the appressorium. In 1 test, an appressorium was formed in 4 hours and infection hyphae from the substomatal vesicle in 8 hours. Such observations, however, do not indicate accurately when the fungus is beyond the reach of ordinary environmental influences, which might be considered the critical end point of the incubation process.

Since high humidity is necessary for infection, and hence for successful incubation, it is a simple matter to determine how long an incubation period is necessary for infection by transferring inoculated plants from a moist incubation chamber to a dry environment after different periods. A dry environment on the leaf surface was insured by exposing the plants to an electric fan on removal from the incubation chamber. As another method of determining whether or not the fungus had established itself within the host, the onion plants were sprayed after specified incubation periods with fungicides known to be toxic to the fungus. This spray treatment should kill all the fungus germ tubes on the surface

TABLE 15

ERADICANT TREATMENTS FOR ONION DOWNY MILDEW WHEN PLANTS WERE REMOVED FROM MOIST CHAMBER AFTER SPECIFIED INCUBATION PERIODS AND DRIED OR SPRAYED TO KILL THE FUNGUS

Time of inoculation, temperature during test, and treatment on removal from moist chamber	2 hours from inoculation to treatment		3 hours from inoculation to treatment		4 hours from inoculation to treatment		5 hours from inoculation to treatment		7 hours from inoculation to treatment		12 hours from inoculation to treatment	
	Plants	Infection	Plants	Infection	Plants	Infection	Plants	Infection	Plants	Infection	Plants	Infection
	number	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
7 p.m. August 14, 1936:												
Dried	9	0	..	..	8	0	10	10	9	11	10	60
Sprayed with 2 per cent lime-sulfur + spreader	9	0	..	..	10	0	11	0	10	20	9	67
Sprayed with 1 per cent bordeaux + spreader	9	0	..	..	12	0	14	0	8	0	9	55
4 p.m. September 3, 1936, at 17° to 15° C:												
Dried	..	..	8	0	..	..	10	0	12	42	14	71
Sprayed with 2 per cent lime-sulfur + spreader	..	..	10	0	..	..	9	0	9	67	16	12
Sprayed with 1 per cent bordeaux + spreader	..	..	7	0	..	..	12	0	9	67	15	66
5 p.m. September 23, 1936, at 18° to 13° C:												
Dried	..	..	..	..	7	71	..	..	7	71	7	85
Sprayed with 2 per cent lime-sulfur + spreader	..	..	..	..	7	86	..	..	9	78	10	90
Sprayed with 1 per cent bordeaux + spreader	..	..	..	..	6	100	..	..	11	72	6	100
9 a.m. October 14, 1936, 13° C:												
Dried	..	..	14	29	9	100	9	100	..	..	..	..
Sprayed with 2 per cent lime-sulfur + spreader	..	..	9	0	8	100	12	100	..	..	..	..
12 m. April 15, 1937, 13° C:*												
Dried	28	0	25	0	36	0	..	..	30	23	28	54
Sprayed with 2 per cent rosin lime-sulfur	30	0	24	0	25	0	..	..	30	0	..	..
9 a.m. June 7, 1937, 13° C:*												
Dried	22	0	..	..	32	0	34	0	82	11	..	..
Sprayed with 2 per cent rosin lime-sulfur	29	0	..	..	38	0	30	0	98	24	..	..

* Seedling plants were used in these tests, all previous tests were with plants grown from bulbs.

of the leaf, and it was thought that such fungicides might follow the germ tubes into the stomata and kill the fungus even long after the latter was beyond the reach of the killing action of dry air. Tests of the use of these eradicant methods to determine the incubation period of the onion downy mildew, summarized in table 15, show considerable variation but indicate that the fungus is beyond the reach of injury by drying in 3 to 7 hours, and that lime-sulfur and bordeaux-mixture sprays were no more effective than drying in preventing infection in such tests.

Rate of Growth of Mildew Mycelium in Onion Leaf.—To determine the rate of growth of onion-mildew mycelium in the leaf, blocks of plain agar and potato dextrose agar bearing sporangia were applied to marked positions on onion leaves, and the inoculated plants were incubated overnight in a moist chamber. Eleven days after inoculation these plants, in a test of February 10, 1938, were incubated in a moist chamber, and the extent of sporulation above and below the point of inoculation was measured.

Of 15 leaves inoculated with single blocks of plain agar bearing sporangia, all showed luxuriant sporulation. The total length of the sporulating surface on the leaf averaged 43 mm. This sporulation averaged 17 mm above the point of inoculation with a minimum of 7 mm and a maximum of 28 mm. The length of sporulating surface below the point of inoculation averaged 26 mm with a minimum of 15 mm and a maximum of 33 mm. The length of the sporulating surface below the point of inoculation for 13 of the 15 leaves was greater than the length above, and for all 15 averaged 53 per cent greater. The extent of the mycelium in the leaf tissues beyond the limit of sporulation was not measured in this test, but in the other tests it has ranged from 15 to 22 mm and averaged 18 mm below the lower limit of sporulation. By assuming the value of 18 mm in this test, the rate of growth of mildew mycelium along a leaf would be about 3.2 mm per day, or 134μ per hour distally, and 4.0 mm per day, or 167μ per hour proximally, or a total extension of about 7.2 mm per day, or 300μ per hour in both directions. This rate of growth is about twelve times that of germ tubes from single sporangia as reported for the agar cultures.

In the test with sporangia on blocks of potato dextrose agar, 20 leaves were inoculated. Eleven days after inoculation only 8 of these showed sporulation, and the extent of the sporulating surface averaged 27 mm per leaf with a minimum of 14 and a maximum of 24 mm. The success of the inoculations and the rate of growth of the mycelium was therefore less from sporangia seeded on potato dextrose agar than from those on plain agar.

Three tests were made to determine if fungicide sprays applied to the surface of leaves 24 hours after inoculation affected the rate of growth of the mycelium in the leaf. In a test of January 5, 1938, leaves inoculated by means of plain agar blocks containing sporangia were treated with a brush below the point of inoculation with 2 per cent rosin lime-sulfur or 1 per cent bordeaux plus 0.5 per cent sulfonated miscible oil, and the extent of the mycelium after 19 days was followed by staining the cleared leaves. In this test the extent of the mycelium in 8 control leaves averaged 50 mm below the point of inoculation, in 3 leaves treated with rosin lime-sulfur 40 mm, and in 5 leaves treated with bordeaux mixture 47 mm. In a similar test of February 17, 1938, and harvested 11 days after inoculation, the extent of the mycelium in 11 control leaves averaged 46 mm below the point of inoculation, in 6 leaves treated with rosin lime-sulfur 47 mm, and in 5 leaves treated with bordeaux 47 mm. In a test of March 1, 1938, the extent of growth of the fungus was followed by measurement of the sporulating surface only (probably unreliable for such tests), and 0.2 per cent malachite green plus 0.2 per cent sodium oleyl sulfate was included in the fungicides tested. In this test the extent of the sporulation was 32 mm below the point of inoculation for 8 control leaves, 16 mm for 4 leaves treated with rosin lime-sulfur, 20 mm for 5 leaves treated with bordeaux, and 34 mm for 3 leaves treated with malachite green. According to these last 3 tests there was no certain effect of the external applications of these fungicides on the internal rate of growth of onion-mildew mycelium. According to tests to be described later (p. 667), however, these materials used as sprays did apparently increase the yield of infected plants.

Infection Period about 7 Days.—To determine the time from inoculation until the fungus is sufficiently mature to sporulate, inoculated plants were incubated in moist chambers each night after inoculation. The minimum period from the night of inoculating the plants to the night of first sporulation was 5 days (June 4, 1936), but in this case only a very few sporophores were formed. The minimum period from inoculation to luxuriant sporulation was 8 days. To determine whether or not plants were infected in most routine inoculation and fungicide tests, they were generally incubated 10 to 12 days after inoculation. At 10 days after inoculation, symptoms usually were not or were only barely evident. In many cases of young infections, sporulation had occurred on plants on which I could detect no symptoms. In the field also, luxuriant sporulation may occur on tissues showing no symptoms. This is contrary to the situation with the downy mildews of hop, cucumber, and snapdragon, with which diseases I have been able to observe symptoms before sporulation could be induced.

EPIDEMIOLOGY

The epidemiological factors I would suggest as critical in determining the incidence and severity of downy mildew on onions in California, are, in order of importance: source of inoculum, temperature, moisture conditions, and wind. It is, of course, impossible to establish an adequate factual basis for these suggestions on my limited field observations.

During 1935 to 1940, onion downy mildew had apparently not become conspicuous or abundant on onions grown for seed in the Cotati, Sacramento—San–Joaquin delta, and southern Santa Clara Valley regions until the spring months, if at all, whereas it usually appeared earlier at Milpitas, and had been found at all seasons in the truck-garden districts of San Francisco Bay region. I believe these differences between districts are due in part to relative abundance of inoculum. In the first three districts mentioned, heavy and early infection would occur only if there was considerable systemic infection in the bulbs used as planting stock, and there usually is not. The infection which usually does appear later in the season probably arises from a few systemic infections, from oöspores in soil or refuse, or from sporangia wind-borne from other districts. At a seed farm near Milpitas, one of the reasons the disease develops earlier and with greater severity than in the other seed-producing localities is probably the large number of varieties of varying degrees of susceptibility, some of which probably always carry some systemic infection. Onions are planted earlier at Milpitas than in the other districts and this might be responsible for the greater spread of the disease. Local weather conditions at Milpitas may be especially favorable to the disease. In the truck gardens of the San Francisco Bay region, where onions are grown throughout the year, principally for greens, the source of inoculum of new plantings is probably principally the sporangia produced on infected earlier plantings.

From the findings of Katterfeld (*31*), Cook (*12*), McKay (*36*), and from data of tables 8, 9, 12, and 14 of this paper, it appears safe to conclude that onion downy mildew is favored by relatively low temperatures, with an optimum temperature of about 13° C, and a maximum of around 25° C. In nature, however, day temperatures are much higher than night temperatures, and it may be possible for onion mildew to thrive with prevailing day temperatures well above the optimum. The relation of prevailing temperatures to the field development of the disease has not been adequately studied.

High humidity at night is necessary for sporulation, and free water is believed necessary for germination and infection, but to what extent

these are limiting to the development of onion mildew under natural conditions has not been determined. Conditions which favor the development of a high humidity on the leaf surface are still air, and clear nights, fog, or rain. Rain, however, might be injurious to the disease in washing the sporangia into the soil. From the observed severity of onion mildew in periods of little or no rain but with abundant dews, I believe rain is of little importance in the development of onion mildew in California, and this belief is supported by the heavy infection which has resulted on plants dusted with dry sporangia and incubated in moist chambers (*84*).

From data of the time required for sporulation, germination, and penetration, Katterfeld (*31*) believed that 1 humid night was sufficient for the production of, and infection with, the same sporangia. From similar data collected by myself, I believe that 2 humid nights are usually necessary—1 humid night for sporulation, 1 day period for dissemination of sporangia, and 1 night with free moisture on the leaves for germination and penetration. Conditions which may delay the drying of the plants after a heavy dew or fog are high humidity, any conditions which interfere with air currents, and rain. A rank growth of weeds may delay the drying of onion leaves and favor mildew infection. At Cotati an isolated patch of wild morning-glory, or bindweed (*Convolvulus arvensis*), in an onion field was apparently responsible for the increased severity of mildew as observed on July 16, 1940. In the area of heavy weed infestation, 99 out of 100 seedstalks showed mildew infection and only 7 out of 100 were erect and appeared as if they might mature some seed; in the field beyond the margin of the wild morning-glory, 92 out of 100 seedstalks were infected and 21 out of 100 appeared as if they might mature seed.

Wind is considered important in reducing the severity of onion mildew in the Sacramento—San Joaquin delta region. In the eastern portion of the Delta, a dry north wind may blow for several days at a time, and certain growers believe this wind is effective in checking the disease after it has become established. In the western portion, strong westerly winds may prevail for periods of several days, but no effect of these winds on onion mildew has been suggested. Near Milpitas, winds are less, and onion mildew is more severe than in the Delta region. Wind is also of importance in determining the extent and direction of spread of the disease by means of air-borne sporangia.

Shipley (*60*) and Walker (*75*) have reported that in the Bermuda and the Canary Islands, respectively, mildew was apparently less severe on the southern coasts than on other parts of the islands, and they attribute this to the wind and southern exposure.

CONTROL BY EXCLUSION AND ERADICATION

In Finland (*19*) laws designed to exclude the disease from the country have been passed, but no information on the success of such a control method is available.

Hot-water treatment of the seed to destroy seed-borne infection has been suggested by McWhorter (*37*) and tested by Newhall (*49*), but since there is no good evidence of seed transmission there is naturally no evidence of control by seed treatment, though Muncie (*42*) reported that formaldehyde treatment of onion seed reduced mildew carried on the seed.

Murphy and McKay (*44, 46*) have demonstrated the effectiveness of heat in destroying onion mildew in infected bulbs. In their first report (*44*), 8 hours at 40° C was sufficient to kill out such bulb-borne infections but in their later tests (*46*) 8 hours at 40° C was inadequate. Using bulbs averaging about 20 grams in size and artificially inoculated 9 days before heat treatment on September 13, I attempted to determine the effect of short exposures of the bulbs to 41° C dry heat on the later development of onion mildew. Of 15 control bulbs, 11 grew and 10 showed systemic infection on October 16. Of 14 bulbs exposed to 41° C for 4 hours, 12 grew and none showed systemic infection. Of 14 bulbs exposed at 41° C for 32 hours, 11 grew and none showed systemic infection. Treatments for 8 and 16 hours also showed complete eradication with the dry heat. According to these results, the bulbs were not injured by an exposure to 41° C for a period eight times as long as necessary to destroy the infection, which indicates that the treatment has a wide margin of safety.

Infection in growing plants was also destroyed by dry heat without injuring the plants, but here the margin of safety was narrower than with bulbs. Results of all tests are summarized in table 16. According to these results, an exposure of 4 to 6 hours in the dark at 43° C destroyed the mycelium in systemically infected plants, without killing the plants, and 8 to 10 hours at 37° C destroyed the mycelium in locally infected plants. Plants exposed to 37° C for more than 10 hours were severely injured. Onion foliage is therefore more easily injured by heat than are onion bulbs, and these foliage treatments are probably of no value except for experimental purposes.

Newhall (*48*) has indicated that destruction of top-set onions in small gardens was responsible for the light attack of onion mildew in Marion Township, New York, in 1937.

The destruction of crop refuse and the rotation of land for onion culture are widely recommended for the control of onion mildew, but little information on the value of these procedures is available.

TABLE 16

ERADICATION OF ONION MILDEW BY EXPOSING INFECTED GROWING PLANTS TO HIGH TEMPERATURE, 1938

Type of infection, date of inoculation, incubation period, and temperature of heat treatment	0 hours' exposure (control)		4 hours' exposure		6 hours' exposure		8 hours' exposure		10 hours' exposure		12 hours' exposure	
	Plants	Infection	Plants	Infection	Plants	Infection	Plants	Infection	Plants	Infection	Plants	Infection
	number	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
Systemic infection, June 4, 57 days after inoculation:												
37° C.	4	100	..	..	5	80	4	75	..	..	..	..
43° C.	..	..	4	0	..	..	..	..	..	..	..	..
Systematic infection, July 2, 28 days after inoculation:												
37° C.	6	67	4	100	..	..	..	..	4	25	..	..
43° C.	..	..	..	..	6	0	..	..	..	..	..	..
Systematic infection, July 8, 22 days after inoculation:												
37° C.	16	94	..	..	5	100	..	..	9	45	..	..
43° C.	..	..	9	11	6	0	..	..	..	..	..	..
Local infection, June 13, 2 days after inoculation:												
37° C.	96	94	118	13	40	2	49	0	95*	0	61†	0
Local infection, August 8, 3 days after inoculation:												
37° C.	38	92	..	..	44	91	..	..	57	0	..	..

* Slight injury to onion plants.

† Moderate injury to onion plants. Severe injury resulted on onions exposed for 24 hours at 37° C.

CONTROL WITH FUNGICIDES

Shipley (*60*) was one of the first to consider the means of control of onion mildew. The foliage treatments which he considered most promising as protective measures were dusting with a mixture of freshly burned quicklime and sulfur, and spraying with 0.1 per cent ferric sulfate. There is no record that he actually used these mixtures. In 1897, Jones (*30*) applied standard bordeaux mixture, standard bordeaux plus soap, potassium sulfide, and sulfur dust to onions for mildew control. No mildew appeared, the solutions did not adhere well, and the bordeaux was injurious to the leaves. Whetzel (*76*) reported the use of bordeaux mixture for onion-mildew control in New York but no evidence was presented to indicate that it was effective. Ralph E. Smith (*61*) reported that spraying experiments for the control of onion mildew in southern California were arranged in 1908, but the season was not a favorable one for the disease, and the results were not satisfactory. Hearst (*24*) reported tests of the use of bordeaux with and without stickers, applied once a week and oftener before mildew appeared on the leaves. In 1917, the only year for which results are given, "the whole patch was blighted evenly over treated and untreated rows." Edgerton (*18*) reported trials of up to 11 applications of bordeaux, bordeaux plus distillate, and bordeaux plus nicotine for the control of downy mildew, a white-spot disease, and *Macrosporium* on onion. No data on disease incidence are given but he states that the treatments did not show a significant increase in yield. Katterfeld (*31*) made 7 applications of 1.0 to 1.5 per cent bordeaux in 2 months to one onion plot and left one plot as a control. His results indicated that the bordeaux application decreased the percentage of systemically infected bulbs by 60 per cent, increased the average weight per infected bulb by 39 per cent, and increased the average weight per healthy bulb by 9 per cent. No data on the gross or relative total yield of the two plots are presented. Milbrath (*41*) reported that among the substances tested for onion downy mildew were lime-sulfur, sulfur dust, copper-lime dust, commercial colloidal copper, copper stearate, bordeaux mixture plus stickers, and light summer oils. Of these substances, copper-lime dust and bordeaux mixture plus paper hanger's paste gave most promise in adhesiveness and fungicidal value. Doran and Bourne (*15*) applied bordeaux mixture and copper-lime dust for the control of onion mildew. They report that copper-lime dust injured the plants in 2 out of 3 seasons and that bordeaux mixture caused a slight increase in yield in the absence of both diseases. Investigators in Wales (*23*, p. 277) reported that a "resin-sulfur spray mixture" was superior to all other chemicals tested for spreading properties

on onions, and gave fairly satisfactory results in onion-mildew control, but no details of these experiments are given. Cook (*12*) reported that spraying with bordeaux mixture and dusting with copper-lime dust and sulfur dust failed to give any indication of control of onion mildew. McWhorter and Pryor (*38*) believed that copper sprays of the bordeaux group were neither sufficiently toxic nor selectively toxic to onion mildew to ensure any practical control of onion mildew, even where generous applications were made with efficient stickers and spreaders. They reported inadequate coverage and injury to onions from lime-sulfur. They found malachite green highly toxic to the onion-mildew fungus and suggested the use of a mixture of malachite green and cuprous oxide as a control agent for downy mildews. Newhall (*49*) reported that malachite green completely inhibited germination of the sporangia of onion mildew at dilutions up to 1 to 150,000, while copper sulfate permitted germination at dilutions greater than 1 to 75,000. In 1939, Newhall (*50*) stated that 4 weekly applications of "potash-rosin-lime-sulfur, red copper oxide, and malachite green with a number of wetting agents such as Grasselli spreader, Ultrawet, Santomerse, cottonseed-oil emulsion, and Lethane" failed to hold onion mildew in check. Investigators in New South Wales (*51*) reported that spraying with bordeaux mixture plus fish oil gave good results in the control of onion mildew. These reports of investigations of fungicidal control are so fragmentary, contradictory, and so poorly supported by data, that a reader might appear safe in concluding that fungicidal control of onion downy mildew has never been adequately demonstrated.

In addition to the specific reports of attempts at the fungicidal control of onion mildew, which have been briefly reviewed, many recommendations and generalizations concerning the control of onion mildew have appeared in the literature, but in most cases the basis for these recommendations is not given, and in view of the published experimental evidence the recommendations appear unjustified. For instance, Osmun (*52*) states: "Control methods for onion blight have been worked out." He recommends the destruction of onion refuse, the rotation of onion land, providing conditions favorable to the aeration of the plants, and spraying with 4–4–50 bordeaux mixture. McCallum (*35*) recommends spraying every 10 days with bordeaux mixture plus resin sticker. Sutton and Sons (*64*) state: "In its early stages the mildew may be successfully dealt with by freely dusting the plants with flowers of sulfur when wet with dew, or by the application of sulphide of potassium in the proportion of one ounce to a gallon of water." Several growers in California have expressed the opinion that onion mildew can be partially controlled by applications of sulfur dust, but no supporting evidence is available.

Concerning soil treatments for onion mildew there is little information. Shipley (*60*) cautioned against the use of wood ashes on onions because the potash might favor onion mildew, but certain manufacturers[8] have made claims in farm papers that potassium sulfate controlled onion mildew.

Tests of fungicides for the control of onion mildew started with a field test in 1935, but as greenhouse tests formed a basis for continued efforts in field tests, the laboratory and greenhouse trials will be reported first. Previous reports of this work have been published (*78, 80, 82, 83*).

Toxicity of Spray Fungicides to Sporangia of Onion Mildew.—I have considered that *in vitro* studies of the toxicity of chemicals are not of great importance in testing materials for their protective value for onion downy mildew. While toxicity to the fungus may be a primary prerequisite for any material to be considered as a fungicide, other factors such as host coverage and the effect of the host on the fungicide may be of great secondary importance. And since the final resultant of these three factors is evaluated in a protective test with little more effort than a toxicity test, I have preferred the protective tests, even though the latter are less adaptable to standardization in procedure and evaluation of results. Some basis for these opinions is given in the toxicity data which follow.

The importance of substrate in toxicity studies is well illustrated in the action of sulfur on onion-mildew sporangia. Sporangia in water suspension were added to glass, agar, and leaf surfaces that had been dusted with sulfur, and adequate controls were maintained. After about 24 hours, germination of the sporangia was counted—100 sporangia counted for each treatment in each test—and the results of these tests were as follows:

Substrate on which sporangia in suspension were placed	Number of tests	Average germination, in per cent
Slide	7	85
Slide + sulfur	7	74
1 per cent plain agar	7	78
1 per cent plain agar + sulfur	7	0.14
Normal detached leaves	1	80
Normal detached leaves + sulfur	1	60
Rubbed detached leaves	1	80
Rubbed detached leaves + sulfur	1	2

On glass slides and on normal detached leaves, dusting sulfur (Flotox) had practically no effect on the germination of sporangia, but on plain agar and on leaves that had been rubbed so that water would adhere

[8] An advertisement in Pacific Rural Press 132:266. 1936.

better, sulfur dust was highly toxic to the sporangia. On sulfur-dusted agar plates to which sporangia had been added, hydrogen sulfide was present in large amounts as indicated by the darkening of lead acetate paper but no hydrogen sulfide was detected from the plain agar without sulfur or from sulfur-dusted slides to which a spore suspension had been added. The effect of rubbing the leaves was presumably to allow a more intimate contact between leaf, sulfur, water, and sporangia.

TABLE 17

EFFECT OF SUCROSE, PEPTONE, AND ASPARAGINE ON THE TOXICITY OF BORDEAUX MIXTURE AND ROSIN LIME-SULFUR TO ONION-MILDEW SPORANGIA

Fungicide suspension	Test-antagonizing substance added to fungicide suspension			
	Germination* in water, control	Germination* in 1 per cent sucrose	Germination* in 1 per cent peptone	Germination* in 1 per cent asparagine
	per cent	*per cent*	*per cent*	*per cent*
Control, water	75	82	87	85
Bordeaux:†				
0.3 per cent	..	..	..	0
0.1 per cent	..	..	0	65
0.03 per cent	..	..	62	74
0.01 per cent	0	0	66	.
0.001 per cent	17	12	..	.
0.0001 per cent	81	68	..	.
Rosin lime-sulfur:‡				
0.01 per cent	0.2	0.0	0.2	0.0
0.001 per cent	44	29	38	42
0.0001 per cent	57	51	53	43

* 100 sporangia were counted in each test and each value is the average of 2 to 6 tests (mostly 4 to 6) on different days.

† Percentage is given as per cent by weight of each constituent. This mixture therefore contained 0.3 per cent $CuSO_4.5H_2O$ (0.077 per cent copper) and 0.3 per cent $Ca(OH)_2$.

‡ Percentage given is per cent by weight of rosin, which was used as rosin soap containing 25 per cent rosin. The lime-sulfur was present in the ratio of 1 volume of lime-sulfur to 1 volume of rosin soap. This mixture therefore contained 0.04 per cent rosin soap and 0.04 per cent liquid lime-sulfur.

Antagonism of Peptone and Asparagine for Copper.—The work of Kunkel (*33*) on the antagonism of peptone to nitrates has emphasized the possible role of antagonism in toxicity tests. The effect of sucrose, peptone, and asparagine on the toxicity of bordeaux mixture and rosin lime-sulfur was measured in 6 tests, which are averaged in table 17. In these tests 3 cc of water or of the test antagonizing agent—sucrose, peptone, or asparagine—at a concentration four times that indicated in table 17 was added to appropriate vials; 3 cc of bordeaux mixture or rosin lime-sulfur at four times the concentration indicated in the table was then added to the appropriate vials. To each vial 6 cc of a spore suspension of *Peronospora destructor* was added, and the vials agitated about every 5 minutes for an hour. Each vial of spore suspension plus test chemicals

was poured onto a separate plate of cold plain agar, allowed to settle about a minute, and the superfluous liquid poured off. After the plates were exposed to dry somewhat for a few minutes they were closed and incubated at 13° C, and the percentage germination of 100 spores on each plate was determined after about 24 hours. The results presented in table 17 show that the critical concentration (highest concentration tested which allowed germination) of bordeaux was increased from 0.01 per cent without any antagonizing agent to 0.1 per cent with 1 per cent peptone, and 0.3 per cent with 1 per cent asparagine. Sucrose had no apparent effect on the toxicity of bordeaux, while none of the materials tested apparently affected the toxicity of rosin lime-sulfur. These tests were not followed with adequate tests of the role of antagonism on onion leaves.

Greenhouse Methods of Testing Protective Fungicides.—All greenhouse tests of fungicides were performed with plants grown in 4-inch pots. In the early tests, plants were grown from bulbs of an unknown but highly susceptible variety secured in the market. In most of the fungicide tests, seedlings of Australian Brown with 10 to 60 plants per pot were used as test plants, and the inoculum was grown on large plants grown from bulbs. Even though field control was primarily a problem of protecting the seedstalks, all the greenhouse tests were on onion foliage. Plants were sprayed with an atomizer using 35 pounds air pressure, or dusted with a hand duster. Dilute sprays, concentrated sprays (or vapor-dust sprays, also called fogs), or dry dusts were used.

With the ordinary dilute sprays the plants were held at about 10 inches from the atomizer and sprayed till runoff occurred. This gave a conveniently observed end point. With vapor-dust sprays or with dry dusts, standardized application was more difficult, for protection from these vapor-dust sprays could be secured from deposits which could be seen only with difficulty. Most of the dosages of vapor-dust sprays were governed by timing, and an exposure of plants for 4 seconds to vapor dust at about 2 feet from the nozzle gave a satisfactory protective coating with several sprays. In most of the tests, some of the sprayed plants were subjected to a weathering test soon after the spray had dried on the leaves. In some tests previously reported (*80*), sprayed plants on which the fungicide had dried were subjected to weathering from natural rain, and its effect on protection was determined. Rain was very unreliable in occurrence, however, and in most later tests the sprayed plants were subjected to artificial weathering by spraying them with water. This was not compared with natural weathering in its effect on protection in the same test, but artificial weathering caused a marked reduction in the protective action of several sprays and is believed to be fairly

comparable to rain. After an arbitrary weathering period the plants were dried, and later inoculated by spraying them with a suspension of sporangia of *Peronospora destructor,* and the inoculated plants were incubated overnight in a moist chamber. The following morning the plants were placed on a greenhouse bench where the prevailing temperatures usually varied from around 20° C during the day to 15° C at night. Ten to 12 days after inoculation the plants were incubated overnight in moist chambers to induce sporulation, and infection was recorded as the percentage of inoculated leaves sporulating for plants from bulbs, or percentage of plants sporulating for plants from seed. In each test 1 or 2 pots of plants not treated with a fungicide were maintained as controls, and if these did not show over 30 per cent infection the results of the test were not used. Infection failures occurred occasionally during the summer months.

Fungicides Used in Spraying Tests.—Bordeaux mixture was prepared by adding 10 per cent stock solutions of copper sulfate and quicklime in equal proportions to the required amount of water, and the concentration is expressed as percentage by weight of copper sulfate. Rosin soap was prepared from the following:

Ingredient	Parts by weight
Water	68
Potassium hydroxide	5
E-grade lump rosin	25
Alcohol	2

In earlier preparations the alcohol was omitted. Later the water, potassium hydroxide, and rosin were heated together to form a soft soap, and the alcohol was added. Commercial liquid lime-sulfur with a specific gravity of 1.26 was used. Rosin lime-sulfur was prepared by adding first the required amount of rosin soap and then the same volume of lime-sulfur to the water, and the concentration is expressed as the percentage by volume of each ingredient. The cottonseed oil and other vegetable- and animal-oil emulsions were prepared by emulsifying 1 pint of oil with 1 egg in a mayonnaise mixer. The self-emulsifying cottonseed oil contained approximately 85 per cent cottonseed oil and 15 per cent phthalic glyceryl alkyd resin. Most of the other fungicides tested were proprietary products.

Relation of Leaf Growth to Fungicidal Protection.—Onion leaves and seedstalks elongate principally at the base, and the younger leaves may grow very rapidly. This growth from the base makes protection with fungicides more difficult because infection on the newly exposed tissues may girdle the leaf or stalk at that point and eventually kill all tissues

beyond, whether or not they are covered with a protective fungicide. No tests were made to determine if infection actually takes place on the newly exposed tissues at the base of the leaf or seedstalk, but such is to be expected, and seems to be the most natural explanation of the results of table 18.

The rate of growth of onion leaves was ascertained by successive measurements of the same leaves in 3 tests. In one of these (table 18) all the leaves of one plant grown from a bulb were marked with ink at the

TABLE 18

REGION OF GROWTH OF ONION LEAVES, GREENHOUSE, DECEMBER 3–10, 1936

Leaf group and region of growth	Increase in length of leaves, per leaf					
	After 2 days		After 5 days		After 7 days	
	Average	Maximum	Average	Maximum	Average	Maximum
	mm	*mm*	*mm*	*mm*	*mm*	*mm*
Leaves which showed growth below point of emergence (7 leaves):						
Below point of emergence	16	22	44	62	64	90
Above point of emergence	6	11	11	22	16	25
Leaves which showed no growth below point of emergence (3 leaves):						
Above point of emergence	3	10	5	15	7	20
Average (10 leaves):						
Below point of emergence	11	..	31	..	45	..
Above point of emergence	5	..	10	..	12	..

point where they emerged from or were attached to the leaf sheath, and the total length of each leaf was carefully measured. Measurements made 2, 5, and 7 days later showed that the 3 outer leaves made no growth below but averaged 7 mm above the initial mark. Seven leaves showed four times as much growth below as above the point of emergence.

In 2 other tests the rate of leaf growth and its apparent effect on fungicidal protection were followed simultaneously. On each of several days, 2 plants grown from bulbs were sprayed with 1 per cent bordeaux plus 0.5 per cent sulfonated miscible oil. This was an effective protective spray which left a conspicuous deposit. As the sprayed leaves grew, unsprayed leaf tissue was exposed below the point at which the leaves emerged from the leaf sheath at time of spraying, and was easily distinguished by the absence of spray deposit. All sprayed plants and 2 control plants were inoculated simultaneously after measuring the leaf growth of the sprayed plants. The results on leaf growth and infection are given in table 19. The values for leaf growth are extremely variable because each

value represents one set of measurements on a different set of 2 plants from each other value, but leaves of individual plants grew as much as 5 cm in 2 days. Infection of sprayed leaves increased with the amount of leaf growth, but in the test of April 10 there was no apparent increase in infection from 3 to 15 days. This is probably because only some of the younger leaves showed any considerable growth and the older leaves had already reached their maximum growth. The 2 tests of table 19 were

TABLE 19

RELATION OF LEAF GROWTH TO THE ACTION OF A PROTECTIVE FUNGICIDE* FOR ONION DOWNY MILDEW, 1936

Date of test and period from spraying to inoculation	Total leaves	Leaves showing growth	Average growth of leaves showing growth	Maximum growth of leaves	Leaves infected
	number	*number*	*mm*	*mm*	*per cent*
April 10:					
0 days	22	..	..	..	0
3 days	7	..	..	6	43
5 days	17	..	..	50	41
7 days	15	..	..	55	60
9 days	15	..	..	87	47
11 days	9	..	..	80	44
12 days	8	..	..	67	50
15 days	13	..	..	142	54
Not sprayed	11	..	..	..	100
May 3:					
0 days	11	..	..	..	0
1 day	11	4	11	12	0
2 days	16	9	25	50	6
3 days	12	7	35	50	8
6 days	10	6	102	150	40
Not sprayed	9	..	..	..	89

* Combined spray of 1 per cent bordeaux + 0.5 per cent sulfonated miscible oil spreader. There was no control on the inherent deterioration with time of the bordeaux in this test, but other tests have indicated that deterioration of the bordeaux could not be responsible for these results.

performed before the tests of table 18, and in the tests of table 19 I did not know or take into account the fact that a small amount of growth may occur above the point of emergence of the leaves from the leaf sheath.

Wetting Agents for Onion Leaves.—Onion leaves are not readily wetted by ordinary solutions or water suspensions, and this difficulty of wetting increases the difficulty of depositing a protective covering on the leaves. To compare the wetting capacity of onion leaves with that of other foliage, the tests summarized in table 20 were performed. The weighed test leaves were held in a vertical position and sprayed with water from a compressed-air atomizer held at a distance of about 16 inches, with a resultant spray of very low impact pressure. Spraying was continued until runoff had just started, and presumably the peak

of deposit had been reached and just passed. The percentage leaf area covered with water was estimated and the leaf again weighed to determine the initial deposit. The leaf was then shaken twice to remove all but the tightly adhering water, the area covered with water was again estimated, the leaf again weighed, and its area measured. Four determinations were made for onion and two for each surface of the other leaves.

TABLE 20

COMPARATIVE DEPOSIT OF WATER SPRAY ON THE LEAVES OF VARIOUS PLANTS, MARCH 21, 1941

Kind of leaf and leaf surface	Estimated leaf area covered with water		Measured deposit of water per square decimeter of leaf surface	
	Before shaking	After shaking	Before shaking	After shaking
	per cent	*per cent*	*grams*	*grams*
Onion (4 determinations)	5	2	0.44	0.07
Pinto bean, primary:				
Lower (2 determinations)	55	50	1.32	0.53
Upper (2 determinations)	70	62	1.59	0.47
No. 45 cantaloupe:				
Lower (2 determinations)	45	7	3.35	0.88
Upper (2 determinations)	30	2	2.02	0.29
Gravenstein apple:				
Lower (2 determinations)	55	10	3.76	1.13
Upper (2 determinations)	35	10	2.24	0.38
Potato:				
Lower (2 determinations)	85	85	1.47	0.64
Upper (2 determinations)	50	35	0.96	0.36
Tobacco (*Nicotiana glutinosa*):				
Lower (2 determinations)	50	12	1.76	0.57
Upper (2 determinations)	60	50	1.41	0.54

With onion almost any type of handling or rubbing greatly increases the wettability of the leaves, and consequently care was taken that the leaves were handled as little as possible before spraying. High impact pressures also greatly increase the wetting capacity of water on onions, and leaves held in a vertical position retain less water than leaves in a horizontal position. These tests were therefore performed under conditions favoring a low deposit on onions, but the tests on the other foliage were performed under similar conditions and on the same day. According to table 20, when the spray is not reduced by shaking and when onion leaves are compared with the average of upper and lower leaf surfaces of other plants, onion leaves will retain only about 30 per cent as much as bean leaves, 16 per cent as much as cantaloupe, 15 per cent as much as apple, about 36 per cent as much as potato, and 28 per cent as much as

tobacco. After shaking, the relative deposit on onions was even less. However, the estimated percentage of the leaf area covered with water, though subject to greater error, is probably more useful than the measured deposit in comparing the different types of foliage. The estimated fractional area covered by water on onion was less than 10 per cent of that on the other foliage, before and after shaking. The apparent discrepancy between estimated percentage leaf area covered and measured

TABLE 21

GREENHOUSE TESTS OF SPRAY SUPPLEMENTS AS PROTECTIVE FUNGICIDES FOR ONION DOWNY MILDEW

Spray material	Coverage	Not weathered		Weathered	
		Tests	Infection	Tests	Infection
		number	*per cent*	*number*	*per cent*
Control, not sprayed	.....	50	87	26	87
0.2 per cent sulfonated miscible oil	Fair	2	59	2	87
0.5 per cent cottonseed-oil emulsion	Fair	4	46	3	94
2.0 per cent cottonseed-oil emulsion	Fair	4	44	4	77
0.5 per cent corn-oil emulsion	Fair	1	76	1	83
0.5 per cent palm-oil emulsion	Fair	1	6	1	63
0.5 per cent coconut-oil emulsion	Fair	1	85	1	33
0.5 per cent castor-oil emulsion	Fair	1	80	1	33
1.0 per cent tank-mix spray oil No. 1	Fair	1	24	1	64
2.0 per cent miscible oil No. 1	Fair	2	38	2	74
1.0 per cent rosin soap	Excellent	4	16	0	—
4.0 per cent rosin soap	Excellent	10	8	3	56
0.2 per cent rosin fish-oil soap	Excellent	1	4	1	78
0.2 per cent sodium oleyl sulfate	Excellent	1	3	1	94
0.1 per cent sodium oleyl sulfate + resin sticker	Excellent	8	16	7	69
0.1 per cent sodium monosulfonate of butylphenylphenol	Excellent	2	0	1	91
0.2 per cent sodium monosulfonate of monobutyl diphenyl	Excellent	2	0	2	52
0.2 per cent ester of a sulfonated bicarboxylic acid	Excellent	1	0	1	100
0.2 per cent sodium salt of an alkyl naphthalene sulfonic acid	Excellent	1	0	1	88

deposit is due to the type of water coverage on the different foliage types. On onion and cantaloupe, the retained water was mainly in the forms of spherical drops, whereas on the other foliage the drops tended to spread out and adhere closely to the leaf surface. Potato was outstanding as a foliage readily wetted with water under these conditions. It is also outstanding as a crop with which spraying has been very successful for the control of a downy mildew, *Phytophthora infestans* (Mont.) D. By.

Onion leaves and seedstalks are readily wetted when certain supplements are added to water or fungicide mixtures used as sprays. Wetting agents added to a fungicide may reduce the maximum deposit on the

leaves to a value much lower than that without the spray supplement, and yet may increase the protective value of the spray (*80*). In addition to improving the coverage of conventional protective fungicide sprays, these supplements in themselves may exert a protective action against onion mildew. Data on the relative coverage and protective action of several spray supplements are given in table 21. Most of the materials tested showed a protective action against onion mildew on unweathered leaves, and this protection was more pronounced with the efficient wetting agents than with the oil emulsions. This protective action may have been due to the direct toxic effect of the supplement on the mildew sporangia, to the action of the supplement in reducing water deposit, or to both. Sporangia of onion mildew failed to germinate in 0.1 per cent sodium oleyl sulfate, but the toxicity of the other materials was not tested. In field tests, plants that had been sprayed with rosin soap and were later subjected to heavy dews at night, dried off in the morning as much as 3 hours earlier than unsprayed control leaves of plants. In greenhouse tests, leaves of plants sprayed with some of the wetting agents were sometimes dry when the inoculated plants were removed from the moist chambers the morning after inoculation, while the unsprayed inoculated controls were still covered with a fine mist of water such as that applied with the inoculum the previous evening. The effect on the infection process of the action of some spray supplements in preventing dew or water spray from adhering to leaves as fine drops was not studied, but it is believed it might be important.

With the exception of coconut-oil and castor-oil emulsions, with which sprays the results are probably atypical, the protective action of all spray supplements was greatly reduced by artificial weathering. On this basis their protective action is clearly distinguished from their action when combined with a conventional protective fungicide (see table 22).

Greenhouse Tests of Protective Fungicides.—Several copper and sulfur fungicides were combined with several spray supplements at various concentrations in several tests. Most combinations were apparently compatible. Malachite green formed a gummy precipitate with several supplements, but the mixture formed when malachite green and sodium oleyl sulfate were mixed dilute was the least objectionable. The addition of sodium oleyl sulfate + resin as a spreader to red cuprous oxide caused the cuprous oxide particles to stick together in groups. Results with most of the spray combinations tested are summarized in tables 22 and 23. Bordeaux mixture without supplements spread much better than water but still gave unsatisfactory coverage or protection. The most effective supplements for bordeaux were several vegetable oils

TABLE 22

COMPARISON OF SUPPLEMENTS FOR COPPER SPRAYS AS PROTECTIVE FUNGICIDES FOR ONION DOWNY MILDEW IN GREENHOUSE TESTS

Copper fungicide and supplement	Not weathered		Weathered	
	Tests	Infection	Tests	Infection
	number	*per cent*	*number*	*per cent*
1.0 per cent bordeaux:				
None	5	76	0	—
0.2 per cent sulfonated miscible oil	8	4	0	—
0.5 per cent bordeaux:				
None	4	77	4	83
0.2 per cent sulfonated miscible oil	2	12	0	—
0.1 per cent sodium oleyl sulfate	7	5	7	9
0.5 per cent cottonseed-oil emulsion	9	0.3	9	0.3
0.5 per cent corn-oil emulsion	1	0	1	0
0.5 per cent palm oil emulsion	1	0	1	0
0.5 per cent coconut-oil emulsion	1	5	1	0
0.5 per cent castor-oil emulsion	1	0	1	0
0.5 per cent sardine-oil emulsion	1	0	1	0
1.0 per cent miscible oil No. 1	4	22	4	46
1.0 per cent tank-mix spray oil No. 2	2	44	2	29
1.0 per cent tank-mix spray oil No. 3	1	40	1	24
0.25 per cent bordeaux:				
None	2	100	0	—
0.25 per cent sulfonated miscible oil	10	14	0	—
0.25 per cent cottonseed-oil emulsion	3	4	3	6
0.25 per cent sardine-oil emulsion	3	0	3	0.5
0.5 per cent red cuprous oxide:				
None	1	48	1	73
0.1 per cent sodium oleyl sulfate	3	42	3	39
0.5 per cent cottonseed-oil emulsion	3	0	2	0
0.5 per cent castor-oil emulsion	1	0	1	17
0.5 per cent copper zeolite:				
None	4	65	4	83
0.1 per cent sodium oleyl sulfate	5	25	5	65
0.5 per cent cottonseed-oil emulsion	2	0	2	4
1.0 per cent miscible oil No. 1	2	29	2	87
0.2 per cent copper phosphate:				
None	1	96	1	92
0.1 per cent sodium oleyl sulfate	1	98	1	100
0.2 per cent cottonseed-oil emulsion	1	11	1	78
0.2 per cent copper sulfate:				
4.0 per cent rosin soap	4	9	0	—

and sardine oil, though bordeaux with these oils did not show as uniform a coverage as with sulfonated miscible oil or with sodium oleyl sulfate + resin. These same oils appeared to have a similar supplementary value when added to the insoluble coppers, red copper oxide, copper zeolite, and copper phosphate. In most tests the copper sprays (table 22) showed little loss in protective value after weathering, in contrast

with the protective action of some supplements used alone. Three mineral oils added to bordeaux exerted only a slight effect on its protective properties.

This general increased effectiveness of copper by the addition of vegetable oils has also been demonstrated in several tests, not reported here,

TABLE 23

LIME-SULFUR AND MISCELLANEOUS SPRAY MATERIALS AS PROTECTIVE FUNGICIDES FOR ONION DOWNY MILDEW IN GREENHOUSE TESTS

Principal ingredient	Supplement	Not weathered		Weathered	
		Tests	Infection	Tests	Infection
		number	*per cent*	*number*	*per cent*
2.0 per cent lime-sulfur	None	8	22	4	82
0.4 per cent lime-sulfur	0.1 per cent sodium oleyl sulfate	3	35	..	..
2.0 per cent lime-sulfur	0.1 per cent sodium oleyl sulfate	18	23	5	56
0.1 per cent lime-sulfur	0.2 per cent rosin soap	1	7	..	..
0.4 per cent lime-sulfur	1.0 per cent rosin soap	2	15	..	..
1.0 per cent lime-sulfur	0.5 per cent rosin soap	2	0	..	..
1.0 per cent lime-sulfur	1.0 per cent rosin soap	9	0	..	..
2.0 per cent lime-sulfur	2.0 per cent rosin soap	10	0	8	9
2.0 per cent lime-sulfur	0.5 per cent cottonseed oil	2	6	2	25
2.0 per cent lime-sulfur	1.0 per cent miscible oil No. 1	1	2	1	5
1.0 per cent rosin soap	1.0 per cent cottonseed oil	3	40	..	..
1.0 per cent rosin soap	2.0 per cent cottonseed oil	2	0	..	..
1.0 per cent wettable sulfur	0.5 per cent cottonseed oil	4	12	3	40
0.2 per cent tetramethyl thiuram disulfide	None	2	0	2	57
0.5 per cent tetramethyl thiuram disulfide	None	2	0	2	4
0.1 per cent tetramethyl thiuram disulfide	0.1 per cent sodium oleyl sulfate	1	2	1	40
0.2 per cent malachite green	None	3	2	3	84
0.2 per cent malachite green	0.1 per cent sodium oleyl sulfate	2	1	2	2
0.2 per cent malachite green	0.1 per cent ester of a sulfonated bicarboxylic acid	1	0	..	..

of protective fungicides for bean rust, bean powdery mildew, cucumber powdery mildew, and cucumber downy mildew. In these tests, however, one proprietary mineral oil has shown supplementary properties similar to that of the vegetable oils, but this material was not tested with onion mildew.

In the hopes of obtaining more efficient methods of application than by conventional spraying, dusts and concentrated spray mixtures were tested. In one test with red copper oxide dust, 100 per cent infection occurred on the treated plants, and in 2 trials of sulfur dust 94 per cent infection occurred on the treated plants. These results indicated that

dust applications had little promise. The vapor-dust mixtures tested were 5 per cent rosin lime-sulfur; 10 per cent rosin lime-sulfur; 2.5 per cent bordeaux; 2.5 per cent bordeaux + 2.5 per cent cottonseed oil; and 5.0 per cent basic copper sulfate + 5.0 per cent cottonseed-oil emulsion. With the exception of bordeaux without supplement, these concentrated mixtures applied as vapor dusts, showed marked protective properties, before and after weathering.

Effect of Fungicides on Sporulation.—A number of fungicides have the property of inhibiting the sporulation of onion mildew without causing any marked injury to the host. Infected leaves were sprayed and allowed to dry before the plants were incubated overnight in moist chambers. Materials tested as vapors were placed in a 350 cc sealed moist chamber with infected detached leaves. The relative sporulation was rated on an arbitrary scale the following morning.

Some of the dried spray coatings which inhibited sporulation are as follows, in their apparent order of decreasing effectiveness: 2 per cent rosin lime-sulfur, 1 per cent potassium sulfide, 2 per cent lime-sulfur, 0.1 per cent sodium oleyl sulfate, 1 per cent ester of a sulfonated bicarboxylic acid, 2 per cent of a miscible pine oil containing 20 per cent copper resinate, and 0.1 per cent malachite green. Bordeaux mixture, with and without spreaders, rosin soap, and sulfur dust were relatively ineffective, though sulfur dust has been found rather effective in inhibiting sporulation of hop downy mildew (*80*), and Doran (*14*) found that sulfur dust inhibited sporulation of downy mildew in cucumber. In 2 tests rosin lime-sulfur was allowed to remain on mildewed leaves in a wet condition for 1 hour, and then washed off. In these tests the inhibition of sporulation on the night after washing and for 2 nights later was apparent, but not so marked as when the spray was allowed to dry on the leaves. Rosin lime-sulfur was more effective than lime-sulfur alone in inhibiting sporulation. But tests in which rosin soap and lime-sulfur were mixed in various proportions indicated that the effect of the rosin soap was mainly to increase the deposit of lime-sulfur, though rosin soap alone inhibited sporulation slightly.

Among the vapor materials that inhibited sporulation were those from dilute lime-sulfur, benzene, paradichlorobenzene, pine oil, and formaldehyde. Lime-sulfur at 0.1 and 1.0 per cent was more effective in inhibiting sporulation than the vapor from concentrated or 10.0 per cent lime-sulfur. The effect of lime-sulfur was apparently due to the hydrogen sulfide evolved.

Therapeutic Action of Spray Fungicides.—To determine if fungicides could reduce the injury from downy mildew apart from their effect in protecting the plants from infection and inhibiting sporulation, several

tests were made in which infected seedling plants were sprayed with test fungicides 24 hours after inoculation with onion mildew, placed overnight in moist chambers in order to give the fungicide a better chance to act, then returned to the greenhouse bench, and harvested after 10 to 36 days. Control uninoculated plants were similarly treated in most of the tests. Control sprayed plants were necessary in order, in the final interpretation, to separate the effect of the fungicide on the plant from its effect on the disease. The results of 13 tests are summarized in table 24. Each value in the table represents the average yield of 4 pots of greenhouse seedlings. Rosin lime-sulfur, bordeaux + sulfonated miscible oil, and malachite green were chosen for these tests because field and greenhouse tests had indicated they were among the best for protection and for the inhibition of sporulation. In these tests, rosin lime-sulfur decreased the yield of healthy plants by an average of 4 per cent, and increased the yield of infected plants by 10 per cent, or the calculated increase in the yield of diseased plants due to the therapeutic action of the fungicide was 14 per cent. Bordeaux + sulfonated miscible oil increased the yield of healthy plants by 18 per cent and increased the yield of infected plants by 24 per cent, or the calculated increase due to the therapeutic action of the fungicide was 6 per cent. Malachite green + sodium oleyl sulfate decreased the yield of healthy plants by 7 per cent (only 2 tests) and increased the yield of infected plants by 43 per cent, or the calculated increase due to the therapeutic action was 50 per cent. As the average yield of infected unsprayed plants was only 42 per cent of the yield of healthy unsprayed plants in these tests, the therapeutic action of these fungicides on diseased plants was far from sufficient to restore them to yield values shown by the healthy plants. It might be considered that the results reported here might be due to a direct eradicant action of the fungicide in killing the mycelium in the tissues, or in reducing the rate of growth of the fungus, but this was not borne out in tests in which these same materials were tested more specifically for these effects (table 15, and text p. 647). Sporulation injury was not a factor in these tests, because the plants were not incubated in moist chambers to induce sporulation.

FIELD TESTS OF SPRAY FUNGICIDES FOR ONION-MILDEW CONTROL

Field applications before 1938 were made with a knapsack sprayer unless otherwise mentioned. Those in 1938 and 1940 were made with a compressed-air sprayer as manufactured for paint application, and powered with a small gasoline engine. Principally because the spray mechanism can be cleaned more easily, but also because it can be used

TABLE 24

EFFECT OF FUNGICIDES ON THE GREEN WEIGHT OF HEALTHY AND DOWNY-MILDEW-INOCULATED GREENHOUSE ONIONS

Date of test, period from spraying to harvest, and condition of plants	Green weight of tops, per pot			
	Not sprayed	Sprayed with 2 per cent rosin lime-sulfur	Sprayed with 1 per cent bordeaux + 0.5 per cent sulfonated miscible oil	Sprayed with 0.2 per cent malachite green + 0.1 per cent sodium oleyl sulfate
	grams	*grams*	*grams*	*grams*
January 20, 1937, harvested 10 days after spraying:				
Healthy	7.84	6.62	....	....
Inoculated	3.39	3.32	....	....
January 29, 1937, harvested 23 days after spraying:				
Inoculated	2.36	2.96	....	....
February 25, 1937, harvested 25 days after spraying:				
Inoculated	6.30	6.20	....	....
March 5, 1937, harvested 27 days after spraying:				
Healthy	6.93	5.95	5.25	....
January 5, 1938, harvested 32 days after spraying:				
Healthy	11.13	9.93	12.97	....
Inoculated	4.73	5.92	4.82	....
January 8, 1938, harvested 30 days after spraying:				
Healthy	5.53	6.67	5.66	....
Inoculated	2.91	2.33	2.98	....
January 14, 1938, harvested 33 days after spraying:				
Healthy	4.71	4.38	5.48	....
Inoculated	1.91	2.85	2.97	....
January 22, 1938, harvested 37 days after spraying:				
Healthy	4.45	4.23	4.71	....
Inoculated	1.78	1.86	2.81	....
February 5, 1938, harvested 35 days after spraying:				
Healthy	5.73	5.33	6.90	....
Inoculated	2.82	1.94	2.98	....
February 28, 1938, harvested 36 days after spraying:				
Healthy	8.45	7.89	....	6.43
Inoculated	2.47	2.62	....	3.78
March 4, 1938, harvested 30 days after spraying:				
Healthy	2.24	2.35	2.07	2.47
Inoculated	0.95	1.28	1.13	1.75
April 10, 1938, harvested 36 days after spraying:				
Inoculated	4.14	4.84	....	4.78
April 28, 1938, harvested 35 days after spraying:				
Inoculated	4.49	....	....	5.39

for vapor dusting with concentrated spray mixtures, the paint-gun equipment is considered much superior to the knapsack equipment. The engine and compressor for the paint-gun sprayer were mounted on a wheelbarrow, and the compressor was operated at 60 pounds air pressure. Spraying with either type of equipment was done thoroughly by spraying the plants first from one side of the row and then from the other. Onion rows in seed crops were about 36 inches apart, and there was some drift from a sprayed row to the adjacent row. Only alternate rows were treated in some of the 1935, 1936, and 1937 tests but as the drift of spray appeared of little importance, no buffer rows were left in the 1938 and 1940 plots. Except for the 1938 treatments on Early Grano at Milpitas, the replications of the same treatment were randomized throughout the area of treatment. Spraying was usually done in the early morning when there was little wind.

1935 Cotati Plots.—In the first field test, 4 per cent rosin soap, 0.25 per cent cuprous oxide + 2 per cent rosin soap, 1 per cent bordeaux, and 1 per cent of a miscible pine oil containing 20 per cent copper resinate were applied on March 27, April 3, 10, 18, 24, May 1, 8, 15, to single rows of White Portugal onions. At the time of first application, 22 per cent of the plants showed infection, and on April 17 all unsprayed plants showed infection. The first seedstalks were observed on April 3, and the first infection on seedstalks on April 17. The bordeaux mixture spread poorly on the leaves but the other sprays spread satisfactorily. Only the rosin soap and cuprous oxide + rosin soap showed marked protective properties. On May 8, seedstalk infection in the control plots was 50 per cent, in 1 plot sprayed with rosin soap 10 per cent, and in 1 plot sprayed with cuprous oxide + rosin 3 per cent. The plants sprayed with rosin soap only were pale in color and showed definite stunting apparently due to spray injury. On July 17, the number of seed-producing stalks in 50 feet of row for control, rosin soap, and cuprous oxide plots was 195, 198, and 269, respectively. On August 8, the ripe seed heads were harvested from 50 feet of row in each plot and the yield of cleaned seed for this picking was 119 grams (average of 3 plots) for the unsprayed control, 157 grams for the rosin-soap plot, and 373 grams for the cuprous oxide plot. No further yield records were secured because the remainder of the plots were harvested as a group by mistake. These 1935 preliminary results, however, indicated that partial control could be secured from frequent applications of cuprous oxide + rosin soap.

1936 Berkeley Plot.—A field planted with Yellow Bermuda bulbs—a highly susceptible variety—on July 31, 1935, was divided into one series of randomized plots each containing 5 plants per plot, and another series containing 25 plants per plot. Mildew infection was not

TABLE 25

FUNGICIDAL CONTROL OF ONION DOWNY MILDEW ON YELLOW BERMUDA ONIONS, BERKELEY, 1936

Treatment	Plots	Plants infected April 25*	Seedstalks infected June 13		Yield of uncleaned seed per plot	
			Weekly† application	Fortnightly‡ application	Weekly† application	Fortnightly‡ application
	number	*per cent*	*per cent*	*per cent*	*grams*	*grams*
Series 1 (5 plants to each plot):						
Control, no treatment	10	86	..	84	....	7.0
2 per cent of a miscible pine oil containing 20 per cent copper resinate	2	50	11	27	81.4§	32.2§
2 per cent lime-sulfur + 0.2 per cent sodium oleyl sulfate	2	30	31	31	51.2	42.8
1 per cent cottonseed oil + 4 per cent rosin soap	2	10	24	42	37.1	39.0
2 per cent basic copper sulfate + 2 per cent rosin soap	2	0	42	62	20.6	52.5
1 per cent saponified copper resinate	2	30	45	63	5.5	60.9
4 per cent rosin soap	2	10	67	86	6.7	0.0
16 per cent rosin soap¶	2	10	65	63	0.0	26.6
0.25 per cent cuprous oxide + 2 per cent rosin soap	4	25	71	64	16.5	15.4
1.0 per cent cuprous oxide + 2 per cent rosin soap	2	40	47	78	37.3	0.0
0.25 per cent copper sulfate + 4 per cent rosin soap	2	10	80	86	18.0	14.3
1.0 per cent bordeaux + 0.5 per cent sulfonated miscible oil	2	20	83	92	3.8	2.6
Cuprous oxide dust	2	90	..‖	..‖	....‖	....‖
Basic copper sulfate dust	2	90	..‖	..‖	....‖	....‖
Series 2 (25 plants per plot):**						
Control, no treatment	2	..	..	84	....	32.5
2 per cent lime-sulfur + 0.2 per cent sodium oleyl sulfate	2	..	..	60	....	105.0
1 per cent bordeaux + 0.5 per cent sulfonated miscible oil	2	..	..	83	....	50.0
0.25 per cent cuprous oxide + 2 per cent rosin soap	2	..	..	77	....	60.0

* Data for weekly and fortnightly applications included.
† Sprayed October 25, November 7, 14, 21, 29, 1935; January 20, 28, February 4, 15, 25, March 3, 11, 21, 29, April 4, 13, 24, May 4, 1936.
‡ Sprayed October 25, November 7, 21, 1935; January 20, February 4, 25, March 11, 29, April 4, 24, 1936.
§ An average of 56.8 grams per plot = 726 pounds per acre.
¶ Severe injury to plants.
‖ Treatment discontinued because of obvious lack of control.
** Sprayed November 21, 1935; January 20, February 4, 25, March 3, 21, April 4, 24, 1936.

found in the plots until February 15, 1936, and was not abundant until April 13. Spray applications were started on October 25, 1936, in the absence of mildew infection, and the applications were made with a large hand atomizer with a pint jar as a container. In addition to downy mildew, considerable infection with *Botrytis allii* appeared in the plant-

ing, which killed many seedstalks, but there appeared little difference in its severity on treated and untreated plots. The onion plants were not so vigorous as is commonly observed in commercial plantings, but downy mildew and spray injury appeared to be the principal causes of differences between plots. A summary of important results from these tests

TABLE 26

FUNGICIDAL CONTROL OF ONION DOWNY MILDEW ON GREEN ONIONS FOR BUNCHING, BAY FARM ISLAND, 1936–37

Treatment	Leaves sporulating November 23		Green weight of plants in 2 feet of row, average per plot			
			December 12, 1936		January 14, 1937	
	Sprayed October 23, November 3, 12	Sprayed October 23, November 3	Sprayed October 23, November 3, 12, 23, 30	Sprayed October 23, November 3, 23	Sprayed October 23, November 3, 12, 23, 30, December 12, 21, 31	Sprayed October 23, November 3, 23, December 21
	per cent	*per cent*	*grams*	*grams*	*grams*	*grams*
Series 1 (4 plots of each):						
Control, no treatment	81	..	170	...	234	...
1 per cent bordeaux + 0.5 per cent sulfonated miscible oil	10	66	235	181	258	297
2 per cent lime-sulfur + 0.2 per cent sodium oleyl sulfate	51	84	184	201	308	291
Rosin soap + 2 per cent lime-sulfur*	1	49	270	287	384†	367
Series 2 (2 plots of each):						
2 per cent miscible pine oil containing 20 per cent copper resinate	0	54	163	217	...	...
1 per cent rosin soap + 1 per cent cottonseed oil	5	70	153	126	...	...
1 per cent rosin soap + 0.5 per cent lime-sulfur	71	83	209	183	...	...

* In two plots 2 per cent rosin soap was used, in two others 4 per cent. Heavier deposit, better disease control, and more host injury resulted from the use of 4 per cent rosin soap.
† 384 grams per 2 feet of row = about 20,800 pounds per acre.

is given in table 25. The plots were obviously too small for material so variable, and downy mildew was less severe and destructive than on plants of this variety at Milpitas in 1938 and 1940, but the results indicate marked disease control with several sprays, including copper resinate in pine oil and lime-sulfur.

1936–37 Bay Farm Island Plot.—On October 23, 1936, a field of onions grown for bunching on Bay Farm Island showed about 25 per cent of the plants with mildew sporulation. The plants were growing in rows 8 inches apart with an average of 32 plants per linear foot of row. A total of 22 plots, 14 of them consisting of 2 parallel rows 4 feet long and 8 of them consisting of 2 rows 33 feet long, were laid out in a uniform

portion of the planting and sprayed as indicated in table 26. Very little apparent development of the disease occurred between October 23 and November 12, but on November 23, every untreated plant examined showed sporulation. All plants sprayed October 23 and November 3 and 12 showed much less sporulation than the controls, but on plots where the

TABLE 27

EFFECT OF TIME AND FREQUENCY OF APPLICATION OF 2 PER CENT ROSIN LIME-SULFUR FOR ONION-MILDEW CONTROL ON AUSTRALIAN BROWN ONIONS, MILPITAS, 1937
(20 feet of row per plot)

Dates of spray application	Plots	Seedstalks healthy		Cleaned seed per plot
		May 28	August 13	
	number	*per cent*	*per cent*	*grams*
Control, untreated	10	43	9	326 (average of 4 plots)
1 application:				
April 23	1	78	8	.....
April 30	1	40	10	.....
May 7	1	47	4	.....
May 14	1	49	19	.....
May 21	1	38	8	.....
May 28	1	37	20	.....
Total or average for 1 application	6	48	11	.....
2 applications:				
April 23, May 7	1	93	35	.....
April 23, May 21	1	92	22	.....
April 30, May 14	1	44	7	.....
April 30, May 28	1	54	20	.....
May 7, 21	1	68	29	.....
May 14, 28	1	53	38	.....
Total or average for 2 applications	6	67	25	.....
3 applications:				
April 23, May 7, 21	5	68	46	562 (average of 4 plots)
April 30, May 14, 28	1	57	49	.....
Total or average for 3 applications	6	62	47	.....
6 applications:				
April 24, 30, May 7, 14, 21, 28	4	94	65	723* (average of 4 plots)

* 723 grams per plot = 1,158 pounds of seed per acre.

November 12 application was omitted, sporulation was relatively abundant. One sample harvest was made on December 12, 1936, and another on January 14, 1937. The results presented in table 26 show a marked increase in yield from most treatments with the greatest increase from rosin lime-sulfur.

1937 Milpitas Plot.—The 1937 treatments were divided into two groups, one to determine the optimum time and frequency of application and one to compare different materials. In the test of time and frequency of application, rosin lime-sulfur was applied in single applications and

in various combinations of 2 to 6 applications. The results, presented in table 27, indicate no markedly significant optimum time of application though the single May 14 and May 28 applications appear superior

Fig. 12.—Effect of spraying on the control of onion downy mildew and *Macrosporium* on Australian Brown onions. Plant on left from plot sprayed April 24, 30, May 7, 14, 21, 28 with 2 per cent rosin lime-sulfur. Plant on right from unsprayed plot. Photographed August 13, 1937, just after seed heads had been harvested.

to the earlier single applications. Increased frequency of application greatly increased the number of healthy seedstalks, the values being 9 per cent healthy seedstalks with no applications, 11 per cent with 1 application, 25 per cent with 2 applications, 47 per cent with 3 applications, and 65 per cent with 6 applications. Yield records show the same trend,

increasing from 326 grams per plot with no applications to 562 grams with 3 applications, and 723 grams with 6 applications. A plant from a control plot and one from a plot receiving 6 applications of spray are illustrated in figure 12.

In the comparison of materials, all treatments showed marked increases in yield over the untreated plots, and the bordeaux + sulfonated miscible oil and rosin lime-sulfur produced the greatest increases in yield (table 28).

TABLE 28

FUNGICIDAL CONTROL OF ONION DOWNY MILDEW ON AUSTRALIAN BROWN ONIONS, MILPITAS, 1937

(10 feet of row per plot; applications April 24, May 7, and 21)

Spray treatment	Seedstalks healthy		Cleaned seed per plot*
	June 4	August 13	
	per cent	*per cent*	*grams*
Control, no treatment	34	4	113, 113, 113, 85
2 per cent lime-sulfur + 0.1 per cent sodium oleyl sulfate	51	21	198, 226, 142
1 per cent bordeaux + 0.5 per cent sulfonated miscible oil	60	58	198, 198, 397†
2 per cent rosin lime-sulfur	73	47	312, 312, 142‡
2 per cent miscible pine oil containing 20 per cent copper resinate	63	42	142, 227, 170

* These yields were originally recorded to the nearest ounce. This accounts for the apparent identity of several plot yields.
† An average of 264 grams per plot = 847 pounds per acre.
‡ The April 24 application was omitted on this plot.

1938 Milpitas Plot.—The 1938 treatments on Yellow Bermuda were designed to test the effect of frequency of application, to compare materials, and to compare the use of concentrated sprays (vapor dusting or fog spraying) with the use of the more conventional dilute sprays. An attempt was made to apply about the same amount of active ingredients in the light fog applications as in the applications of dilute sprays, and the heavy fog application was about twice as heavy as the light. The results, presented in table 29, indicate that marked control of downy mildew and increase in seed yield resulted from most of the treatments, the best being weekly applications of dilute rosin lime-sulfur spray. A treated and control plot are illustrated in figure 13.

The yield of plants sprayed weekly with rosin lime-sulfur was 58 times that of the control plants. In spite of the data of table 29 it is not safe to conclude that the light applications of concentrated sprays are inferior to the heavy wash applications of dilute sprays. The equipment and arrangement of test plots was not ideal for such a comparison, and a more efficient applicator and larger plots would have been desirable for fog applications.

TABLE 29

FUNGICIDAL CONTROL OF ONION DOWNY MILDEW ON YELLOW BERMUDA ONIONS, MILPITAS, 1938

(10 feet of row per plot)

Treatment	Seed-stalks infected May 24	Erect seedstalks per plot		Yield of clean seed	
		April 27	July 7	Yield of each plot	Average for treatment
	per cent	*number*	*number*	*grams*	*grams*
Control, no treatment	59	68	4.3	1.78, 2.47, 0, 0.56, 2.18, 1.53, 1.66, 1.61, 3.75	1.73
2 per cent rosin lime-sulfur spray:					
Weekly*	25	90	52	52.2†, 129.2, 98.5, 122.7	100.7‡
Fortnightly§	29	88	35	82.6, 8.47, 45.2, 20.4	39.2
10 per cent rosin lime-sulfur fog, light:					
Weekly*	36	82	27	3.25, 120.9, 3.60, 26.4	38.5
Fortnightly§	50	78	11	9.93, 3.12, 5.40, 9.87	7.08
10 per cent rosin lime-sulfur fog, heavy:					
Weekly*	38	64	13	11.5, 3.22, 75.5, 6.34	24.1
Fortnightly§	45	82	14	10.9, 5.27, 4.54, 23.0	10.9
0.25 per cent cuprous oxide + 0.25 per cent cottonseed-oil-emulsion spray:					
Weekly*	19	96	35	24.4, 93.8, 20.6	46.2
Fortnightly§	29	66	8	7.55, 1.09	4.32
2.5 per cent cuprous oxide + 2.5 per cent cottonseed-oil-emulsion fog:					
Weekly*	39	71	14	10.6, 1.78, 15.5	9.29
Fortnightly§	43	66	7	0.40, 7.78	4.09
0.2 per cent malachite green + 0.2 per cent sodium oleyl sulfate:					
Weekly*	20	91	29	69.9, 8.03	39.0
Fortnightly§	45	78	15	10.5, 8.09	9.29
0.25 per cent tetramethyl thiuram disulfide:					
Weekly*	49	102	10	10.5, 7.50	9.00
Fortnightly§	62	105	6	3.34, 3.22	3.28

* Sprayed February 25, March 4, 14, 21, 30, April 6, 13, 20, 27, May 6, 16, 23.
† Several plants were injured by cultivation.
‡ 100.7 grams per plot = 322 pounds per acre.
§ Sprayed February 25, March 14, 30, April 13, 27, May 16.

The 1938 Milpitas plot on Early Grano was designed to compare rosin lime-sulfur with bordeaux and cottonseed oil and to compare spray with fog applications. The area under test consisted of 6 rows 200 feet long. The stand was poor, many of the bulbs having rotted during the winter, but was fairly uniform. The area was divided into 3 sections 67 feet in length, and the 6 rows of the center section were left as a control. At one end of the area, 3 rows were treated with rosin lime-sulfur spray and 3

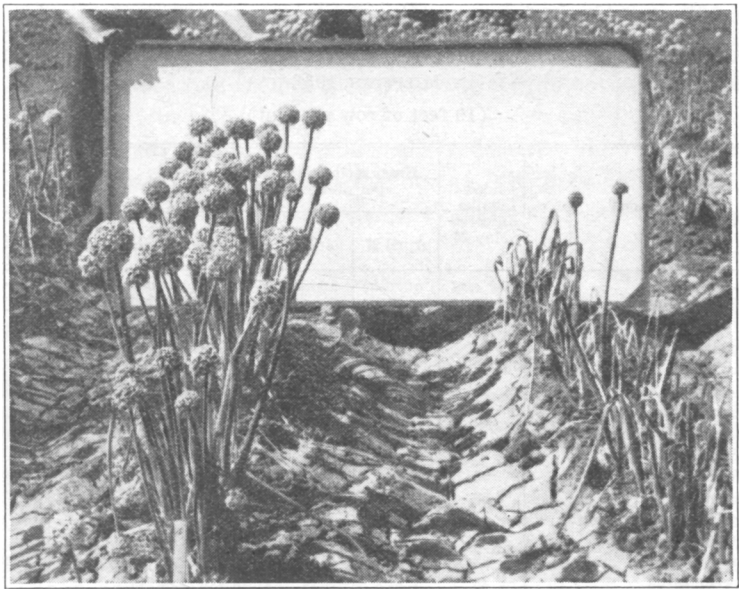

Fig. 13.—Fungicidal control of onion downy mildew on Yellow Bermuda onions at Milpitas, 1938. Plot on left was sprayed February 25, March 4, 14, 21, 30, April 6, 13, 20, 27, May 6, 16, 23 with 2 per cent rosin lime-sulfur. Plot on right was unsprayed.

TABLE 30

FUNGICIDAL CONTROL OF ONION DOWNY MILDEW ON EARLY GRANO ONIONS, MILPITAS, 1938

(Plots 67 feet long, not randomized; poor stand of plants)

Treatment	Plots	Average yield per plot	
		Heads harvested	Cleaned seed harvested
	number	*number*	*grams*
Control:			
0 applications	6	135	133
2 per cent rosin lime-sulfur spray:			
5 applications*	2	172	194
4 applications†	1	147	275
10 per cent rosin lime-sulfur fog:			
5 applications*	2	129	110
4 applications†	1	197	163
0.5 per cent bordeaux + 0.5 per cent cottonseed-oil spray:			
5 applications*	2	151	256
4 applications†	1	88	124
5.0 per cent bordeaux + 5.0 per cent cottonseed-oil fog:			
5 applications*	2	142	299‡
4 applications†	1	140	260

* Applications March 30, April 6, 20, May 6, 23.
† Applications March 30, April 6, 20, May 6.
‡ 299 grams per plot = 142 pounds per acre.

with rosin lime-sulfur fog; at the other end, 3 rows were treated with bordeaux cottonseed-oil spray and 3 with bordeaux cottonseed-oil fog. Downy mildew was only moderately severe, and *Botrytis allii* was moderately abundant, but infection was not counted. The results presented in

TABLE 31

FUNGICIDAL CONTROL OF ONION DOWNY MILDEW ON YELLOW BERMUDA ONIONS, MILPITAS, 1940

(10 feet of row per plot)

Treatment	Plots	Average erect seedstalks, per plot		Yield of cleaned seed per plot	
		May 10	July 15	Yield of each plot	Average for treatment
	number	*number*	*number*	*grams*	*grams*
Control:					
0 applications	10	19.6	1.0	0.40, 0.11, 0.08, 0.66, 0.01, 0.13, 1.43, 0.0, 0.0, 0.05	0.29
2 per cent rosin lime-sulfur:					
Weekly*	5	46.2	9.2	6.30, 19.5, 1.87, 8.76, 3.20	7.92†
Fortnightly‡	5	34.4	8.2	1.33, 0.30, 1.54, 3.94, 2.29	1.88
0.2 per cent cuprous oxide + 0.2 per cent self-emulsifying cottonseed oil:					
Weekly*	5	26.4	2.4	0.28, 0.02, 1.60, 0.25, 0.67	0.56
Fortnightly‡	5	20.4	3.6	0.62, 6.33, 0.01, 1.10, 1.20	1.85
0.2 per cent cuprous oxide + 0.2 per cent self-emulsifying cottonseed oil + 0.2 per cent malachite green:					
Weekly*	5	29.0	3.2	1.79, 3.20, 2.28, 1.58, 0.89	1.95
Fortnightly‡	5	29.0	3.8	0.86, 2.57, 0.17, 0.32, 0.20	0.82
0.5 per cent bordeaux + 0.5 per cent cottonseed oil:					
Weekly*	5	18.8	4.0	2.02, 0.14, 6.91, 0.0, 0.57	1.93
Fortnightly‡	5	16.0	2.6	0.0, 0.65, 1.40, 1.90, 0.22	0.83

* Sprayed March 4, 11, 18, 25, April 1, 8, 15, 22, 29, May 6, 13.
† 7.92 grams per plot = 89 pounds per acre.
‡ Sprayed March 4, 18, April 1, 15, 29, May 13.

table 30, show rather erratic results but indicate a marked increase in yield from most treatments, with the greatest increase in yield from bordeaux cottonseed-oil fog.

1940 Milpitas Plot.—The 1940 Milpitas plot was designed mainly as repetition of previous tests, but more plots of each treatment were used in order to increase the significance of yield differences. Applications were started on March 4, too late for good control because leaf infection was already severe and many seedstalks were already infected. The results summarized in table 31 indicated a marked increase in yield from all treatments, and rosin lime-sulfur spray weekly appeared to be the best treatment.

MISCELLANEOUS OBSERVATIONS ON FIELD TESTS WITH FUNGICIDES

The amount of spray required for onions varies with the size of plants, spacing of rows, wind, spreading properties of spray, and the type of equipment used, and is more than one might expect from the small size and convenient exposure of the leaves and seedstalks. For Yellow Bermuda onions with fully extended seedstalks, about 1 liter of rosin lime-sulfur spray was required per 10 feet of row, or about 320 gallons per acre under the conditions of the experimental tests.

Most sprays caused injury to onions under field conditions, but 2 per cent lime-sulfur + 0.1 per cent sodium oleyl sulfate appeared to be slightly stimulatory to healthy bulb plants at Concord and healthy seed plants at Cotati in 1936. Rosin lime-sulfur caused marked injury to Yellow Burmuda onions, which injury was manifest as a scorching of the seedstalks. But in spite of this spray injury, applications of rosin lime-sulfur caused marked reduction in downy-mildew infection and increase in yield when applied under conditions of heavy infection. Bordeaux mixture seemed to cause a general weakening of Yellow Bermuda plants but no localized injury was observed. Australian Brown onions showed no apparent injury from any sprays tested.

During 1938, 1939, and 1940, a commercial grower sprayed several lots of onions at Milpitas. In 1938 and 1940, 2 per cent rosin lime-sulfur was used, and 0.5 per cent bordeaux + 0.5 per cent cottonseed oil was used in 1939. Though unsprayed controls were not maintained for comparison, mildew control did not appear highly satisfactory, and considerable disease developed in the sprayed plants. In 1939 most of the disease injury was apparently due to *Botrytis allii*. These commercial applications of rosin lime-sulfur and bordeaux + cottonseed oil with high impact pressures, appeared to cause more severe injury to onions than the experimental applications with low impact pressures.

None of the spray treatments was effective in preserving the leaves of plants grown for seed. In all cases of severe infection the leaves were usually killed by blossoming time, though they usually persisted longer on sprayed than on unsprayed plants.

DISCUSSION OF FUNGICIDAL CONTROL OF ONION MILDEW

The results of all successful field tests for onion-mildew control, given in some detail in tables 25 to 31, and summarized briefly in table 32, demonstrate that with a severe infection, plants sprayed frequently with a suitable fungicide will greatly outyield unsprayed plants. Complete or nearly complete control, however, was not secured in any test. The high

incidence of infection was most likely due in part to the severe conditions of these tests. In all cases many unsprayed control plots were maintained, and as these were heavily infected in the successful tests (those in which a high incidence of disease occurred and marked yield increases from spraying resulted), the sprayed plants were subjected to continuous and heavy inoculation. In addition to the untreated controls, many of the treatments were not expected to and did not give as satisfactory

TABLE 32

SUMMARY OF BEST TREATMENTS USED IN FIELD TESTS FOR FUNGICIDAL CONTROL OF ONION DOWNY MILDEW

Date of first spray application, location, and type of crop	Condition of mildew at start of test	Best treatment used			Yield of best treatment as a percentage of yield of unsprayed control plots
		Spray material	Successive applications of same spray	Replications of treatment	
			number	*number*	*per cent*
March 27, 1935, Cotati, for seed	Abundant	Cuprous oxide + rosin soap	8	1	315
October 25, 1935, Berkeley, for seed	Absent	Copper resinate in pine oil	19	2	797
October 23, 1936, Bay Farm, for greens	Abundant	Rosin lime-sulfur	8	4	160
April 23, 1937, Milpitas, for seed	Abundant	Rosin lime-sulfur	6	4	222
February 25, 1938, Milpitas, for seed	Abundant	Rosin lime-sulfur	12	4	5,820
March 30, 1938, Milpitas, for seed	Moderate	Bordeaux + cottonseed oil	5	2	224
April 29, 1938, Sacramento, for seed	Moderate	Bordeaux + cottonseed oil	4	2	No record
March 4, 1940, Milpitas, for seed	Abundant	Rosin lime-sulfur	11	5	2,730

control as others, and the plots showing poor control also were presumably partly responsible for a heavy spore shower on the sprayed plants. Furthermore, in all successful tests but the 1936 Berkeley test and the March 30, 1938, Milpitas test, the experimental block of onions was immediately adjacent to, or only a few feet distant from, a block of unsprayed mildew-susceptible and heavily infected onions.

In addition to 8 successful tests (table 32) in which mildew infection was severe and significant increases in yield due to spray treatment were noted, 5 tests were performed in which practically no increase in mildew occurred after the tests were started. In the test of January 15, 1936, at Concord, healthy plants grown for bulbs were sprayed in duplicate plots with cuprous oxide + rosin soap, bordeaux mixture + spreader, lime-sulfur + sodium oleyl sulfate, rosin soap, copper resinate soap, and precipitated copper resinate on January 15, 30, February 15, and March

3, 17, 31. On March 31 no mildew infection had appeared in the planting and the test was discontinued. The 1 per cent bordeaux mixture + spreader caused slight injury, but the other treatments showed no injury, and plots sprayed with 2 per cent lime-sulfur + 0.2 per cent sodium oleyl sulfate appeared slightly more vigorous than the other treated plots or the untreated controls. In the test of January 22, 1936, at Cotati, plants grown for seed were sprayed with the same materials and at approximately the same time as the January 15, 1936, plot at Concord. This test was discontinued on March 4 because only a trace of mildew had appeared in the planting. In the test of March 19, 1936, at Concord, onions grown for seed and showing moderate infection were sprayed in triplicate plots with bordeaux mixture + spreader and cuprous oxide + rosin soap on March 19, 31, April 10, 21, and May 4. The treatments were discontinued on May 4 and no further records were taken because mildew had apparently not increased since the start of the test. The other unsuccessful tests, one at Sacramento, and one at Concord, were also discontinued because mildew failed to become abundant in the untreated plots during the late spring or early summer months.

Although this report involves several successful field tests of fungicides, the results do not constitute an adequate basis for recommendations for the commercial control of onion mildew with fungicides. The most successful field tests were at Milpitas, where onion mildew appeared earlier, and did more damage than at Cotati, in the Sacramento–San Joaquin Delta region, or in the southern part of the Santa Clara Valley, each of which districts is more characteristic of onion culture in California than Milpitas. A less intensive onion-mildew-control program would very likely be required in these other districts than was necessary at Milpitas.

All sprays were applied with rather miniature equipment in this study, and the problem of proper equipment for applying fungicides to onions was not studied. Equipment built high enough to clear the tops of onion seedstalks would probably be necessary.

So far as this study would indicate, dusting would not give control, and conventional dilute sprays would be most successful, but vapor dusting with ground or air-borne equipment would seem very promising. Several fungicidal sprays gave marked protection against onion mildew and marked increases in yield of onion seed, but which material was best, however, is not obvious. Applications of rosin lime-sulfur were associated with the two highest relative yields in this study, but it was also more thoroughly tested than any other material. Advantages of rosin lime-sulfur over some of the other sprays used are its low cost and its

convenience of preparation if the stock materials are available. Disadvantages of rosin lime-sulfur are the labor of preparing the rosin soap and the tendency of the spray to form gum. Because of the high viscosity of rosin lime-sulfur, preparations containing 10 per cent of each of the liquid components in water are about the maximum concentration which will maintain satisfactory liquid properties, and this difficulty of getting a concentrated spray would probably make the use of rosin lime-sulfur impractical as a commercial fog spray. Bordeaux mixture with cottonseed oil was a promising combination but this has the disadvantage of requiring 4 components, and if the copper sulfate and lime must be prepared as separate stock solutions, the labor of preparation becomes considerable. The bordeaux + cottonseed-oil combination was unsatisfactory on Yellow Bermuda in 1940. Copper resinate in pine oil appeared to be the best material tested in the 1935–36 plot at Berkeley, but in the 1937 plot at Milpitas it was the poorest. This is possibly because the materials used in those two seasons were from different samples with quite different physical properties.

GENERAL DISCUSSION OF ONION-MILDEW CONTROL

The methods that appear most promising for offsetting the danger from onion mildew are: the development of mildew-resistant varieties, the avoidance of disease, the production and storage of sufficient seed in seasons when the disease is not severe to make up for losses during seasons of mildew severity, and the use of protective sprays. The production of mildew-resistant onions has already been discussed.

Avoidance of the disease might be accomplished by several methods. First, only healthy bulbs should be used as planting stock. If bulbs suspected of harboring the disease are to be used, the infection should be killed out by heat treatment. The bulbs should be planted some distance from other onion fields, and contaminated soil or refuse should be avoided. Second, onions should be grown if possible in a location unfavorable for the development of onion mildew. While in general, districts favorable for onion-seed production appear favorable for the development of onion mildew, some, because of local freedom from dew or rain at critical periods may be unfavorable for its development. When one considers that enough onion seed for the United States for one year can be produced by 1,000 to 2,000 acres of healthy high-yielding onions, the problem of avoiding the disease should not be unsurmountable.

The production of sufficient onion seed in seasons when onion mildew is not severe, to make up for losses in mildew-severe seasons is one of the principal methods in practice for meeting the losses from onion mildew. With improved knowledge of optimum storage conditions for onion

seed (*4*), this method might be used with more certainty, but to the individual grower the method involves the risk of loss of a large part of his crop in epidemic seasons.

Protective sprays should be used to supplement the above-discussed control methods. According to the observations and results reported in this study, it would appear inadvisable to apply protective fungicides before the disease is actually present in an onion planting. In several tests reported here, marked control was secured by spraying started after the disease was well established. The critical period for protection appears to be during growth of the seedstalks. If infection has become established before the seedstalks emerge, many applications might be necessary to secure adequate control, but if infection did not become established until the seedstalks had completed their elongation, one application might be sufficient. In this study, spraying dates were arbitrarily chosen about a week apart irrespective of the development of the disease, because frequent observation of the plantings was impractical. In practice, however, it would appear desirable to time the sprays according to the observed development of the disease. Sporulation under field conditions is rather erratic, and spray coverage during periods when no sporulation occurred would be of much less value than sprays applied preceding and following active sporulation.

SUMMARY

Onion mildew was first reported in England in 1841, and is now worldwide in distribution. The disease is most severe on the seed crop, and losses of from 0 to 70 per cent of the California onion seed crop due to onion-mildew infection have been reported. Losses in individual fields vary from none to complete. The leaves, which are usually infected first, are apparently of little importance to the seed crop, but when the seedstalks are infected the yield is reduced.

Onion mildew has been recorded on the following species of onions: *Allium Cepa, A. fistulosum, A. nigrum, A. Porrum, A. ursinum, A. oleraceum, A. sativum, A. ascalonicum,* and *A. Schoenoprasum.* Only *A. Cepa* and *A. fistulosum* have been observed infected in this study. All varieties of the common onion are susceptible, but marked differences exist between varieties in the amount of injury caused by mildew infection.

Characteristic symptoms are the paling, down-curling, and narrowing of the leaves in systemic infections, the large, oval, slightly chlorotic lesions on leaves and seedstalks resulting from secondary infection, and a general killing of the leaf tips. The grayish-violet downy growth of sporangiophores on the surface of infected tissues is the most characteristic sign of the disease.

Peronospora destructor Berk. appears to be the proper name of the organism causing onion downy mildew. A description of the organism and a discussion of its nomenclature is given. Sporangia of *P. destructor* have failed to make continued growth on agar media to which various test nutrients were added. The most stimulatory of the test nutrients was potassium permanganate, dibasic sodium phosphate, glycine, and melted agar cultures of *Phytophthora citrophthora.* Host extracts were toxic in heavy doses and slightly stimulatory in small amounts. On agar plates the germ tubes grew at about 30μ per hour.

Other organisms causing important losses to onions observed in this study were, in order of importance: *Botrytis allii, Macrosporium,* and *Botrytis cinerea.* All were less important than *Peronospora destructor. Macrosporium* infection was more frequent on the north side than on the south side of onion seedstalks.

In California the principal method by which onion mildew is carried over from one season to another is believed to be by means of mycelium in the bulbs. The amount of seasonal carryover is small. Seed from heavily infected plants produced healthy plants in 2 tests.

Sporangia are normally formed at night, matured in the early morning, and liberated throughout the day. The optimum relative humidity for the formation of sporangia was about 100 per cent and the minimum about 90 per cent. Sporulation in low relative humidity caused the sporangiophores to be shorter than at high humidity. The formation of sporangiophores is governed by the alternation of light and darkness in the normal day as well as the relative humidity. The formation of sporangiophores and sporangia causes injury to the infected plants. Sporangia are disseminated by wind.

When attached to sporangiophores on living leaves sporangia remain viable for about 3 days, but when detached and on the surface of healthy leaves sporangia remain viable for only about 1 day.

Germination of sporangia occurs in the presence of free water, and the germ tube enters the host through the stomata by means of an appressorium and a substomatal vesicle. In the process of penetration of onion mildew, nuclei of the adjacent onion epidermal cells move toward the invaded stomata. Germ tubes had penetrated beyond the killing action of drying or eradicant sprays in about 7 hours, and the mycelium grew at the rate of about 300μ per hour in the leaf. Infected tissues sporulated in a minimum of 5 days after inoculation in one test, but this interval was usually longer.

Artificial inoculation was successfully accomplished by a variety of methods. Systemic infection of plants grown from bulbs was induced by injecting a spore suspension into the bulbs before planting them.

The epidemiological factors considered of most importance in determining the severity of onion mildew attacks are inoculum, temperature, moisture conditions, and wind. Onion mildew may be severe in the absence of rain.

Sporangia germinate better on plain agar than in water. Sporangia germinated and caused infection at temperatures from 1° to 28° C with an optimum at about 13° C. Sporangiophores were formed at temperatures from 7° to 22° C.

Downy-mildew mycelium was killed by heating infected bulbs for 4 hours at 41° C and by heating infected leaves for 10 hours at 37° C.

Onion leaves were less readily wetted by water than were other leaves tested, but were readily wetted by water or by water suspensions of fungicides to which certain spray supplements were added.

The action of spray fungicides on onion mildew was studied by several methods. In spore-germination tests, peptone and asparagine were antagonistic to bordeaux mixture. Sulfur dust was toxic to sporangia on agar plates and on rubbed onion leaves, but was not toxic on glass slides or on normal onion leaves. Sulfur sprays were more effective than copper sprays in inhibiting sporulation. Spraying infected plants with rosin lime-sulfur or with malachite green apparently did not kill the mycelium in the tissues but did reduce the injury from infection. The addition of vegetable oils to several copper sprays increased the protective value of these sprays. In all field tests in which onion mildew was severe in the untreated plots, spraying with various fungicides reduced the incidence of disease, and increased yields by 60 to 5,700 per cent in different tests. Rosin lime-sulfur was perhaps the best fungicide mixture tested. Applications of concentrated sprays in the form of vapor dusts gave marked control of onion mildew, but no control was secured with dry-dust fungicides.

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plantings. The spray plots at Milpitas were on the trial grounds of Associated Seed Growers in charge of G. W. Scott, who generously provided many fine facilities for the conduct of these tests. All planting, field care, harvesting, and weighing of these plots was contributed by Associated Seed Growers.

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