GRAY MOLD OF TOBACCO [1]

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INTRODUCTION

The prevalence of tobacco diseases in seed beds in North Carolina was investigated during the seasons of 1928, 1929, and 1930. Among the diseases found was one caused by a species of Botrytis to which little attention has previously been given and to which the name "gray mold" may appropriately be applied. This disease is characterized by a gray moldy coating which covers all affected parts. Apparently no one has studied the gray-mold disease of tobacco seedlings within the United States, although it has been investigated in certain other tobacco-growing regions. This report, therefore, embodies the knowledge of the present status of this disease, especially as regards its history, distribution, etiology, and the conditions governing its prevalence.

HISTORY, DISTRIBUTION, AND IMPORTANCE OF GRAY MOLD

Fragmentary notes constitute the only published accounts of Botrytis as being pathogenic to tobacco. The mention by Sorauer (*6*),[2] in 1886, of Botrytis on seedlings in seed beds in Germany appears to constitute the first account of this disease. De Toni (*12*), nine years later, noted a leaf spot of tobacco in Italy the cause of which he identified as *B. vulgaris* Fr.

In Peters's (*5*) compilation dealing with diseases and insect enemies of tobacco there is mentioned the occurrence in Java of *Botrytis cinerea* Pers. The author states that the stems of seedlings near the surface of the soil are involved in decay. Their roots and those of older plants become decayed, and large spots appear on the leaves. Ultimately the capsules are attacked, from which it is inferred that the fungus may be seed borne. He notes further that it may appear even on cured leaves.

The widespread occurrence of *Botrytis cinerea* on the fading flowers of various cultivated plants is a matter of common knowledge to plant pathologists. Pape (*4*) observed it on flowers of both *Nicotiana tabacum* and *N. rustica* in Germany. Diseased corollas that in falling chanced to lodge on leaves served as the inoculum for leaf spots. This observation has recently been confirmed by Böning (*3*).

Undoubtedly gray mold has existed in North Carolina for years, although no record of its occurrence as a seedling disease has been found. Neither is there any account of its presence in other tobacco-growing sections of the United States. Although it was noted in nearly every seed bed examined in 1928 and 1929, in a few cases only was it of serious consequence. Several seed beds were seen in which

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[2] Reference is made by number (italic) to Literature Cited, p. 174.

gray mold was so abundant and so destructive that no plants were drawn from these beds for transplanting. In some cases the high mortality of plants set in the field was attributed by growers to the fact that the plants bore gray-mold lesions on the stems when they were pulled from the beds.

APPEARANCE OF THE DISEASE

Gray mold in North Carolina is essentially a disease of seedlings that are of sufficient size to be transplanted. It continues to develop throughout the remainder of the season on plants that remain in the beds after the close of the season for transplanting. Affected plants are, of course, usually discarded at the time of removal from the seed beds. If they are overlooked and chance to be transplanted in the field during a rainy period they may succumb; on the other hand, if transplanted in a loose, moist soil during a relatively dry period the larger proportion will completely recover.

The disease first appears on the lowermost leaves, during relatively dry weather, as large, brown, dry spots, bordered by a yellowish zone. During rainy periods wet-rot lesions are formed and the surface of invaded tissues is covered with a grayish coating of conidiophores and conidia. This coating constitutes a characteristic sign of the disease and is infallible as a criterion for diagnosis in the field. In favorable weather the blade is soon completely invaded and dries, though remaining attached, and the leaf collapses. Meanwhile the fungus has grown downward through the petiole into the stem. (Fig. 1.) The resulting stem lesions are dark brown to black and sunken. The size of these lesions appears to be governed by moisture conditions. In dry weather they remain small and the wound may completely heal. In wet weather, on the contrary, the lesion extends around the stem and the top is killed in consequence of this girdling. The blackened areas on stems may become several inches long and may be covered with a dense grayish coating similar to that on affected leaves. (Fig. 2.) If the leaves become detached and no conidiophores are present, the stem lesions may be confused with those produced by the sore-shin fungus, *Rhizoctonia solani* Kühn.

In seed beds abandoned because of the occurrence of this disease, from which no plants are pulled, all of the lower leaves generally become completely invaded and dry. Leaves higher on the stem bear large, dead patches. A similar condition obtains in beds in which the plants are still closely crowded after the season for transplanting is ended. This is especially noticeable in late June and July in beds that have not been plowed up. The etiolation of the older leaves, occasioned by the development of many plants in close proximity, appears to predispose the leaves to attack.

The appearance of affected tissues, as revealed by microscopic examination, shows that the mycelium of the pathogene is intercellular. The hyphae force their way between the cells as a result of their ability to produce a dissolution of the middle lamella. The cells behind the zone which is being invaded collapse and dry rather quickly. (Fig. 3.)

All leaf tissues are equally subject to attack, as shown in Figure 4, F. The extension of the mycelium between the cortical cells of the petiole

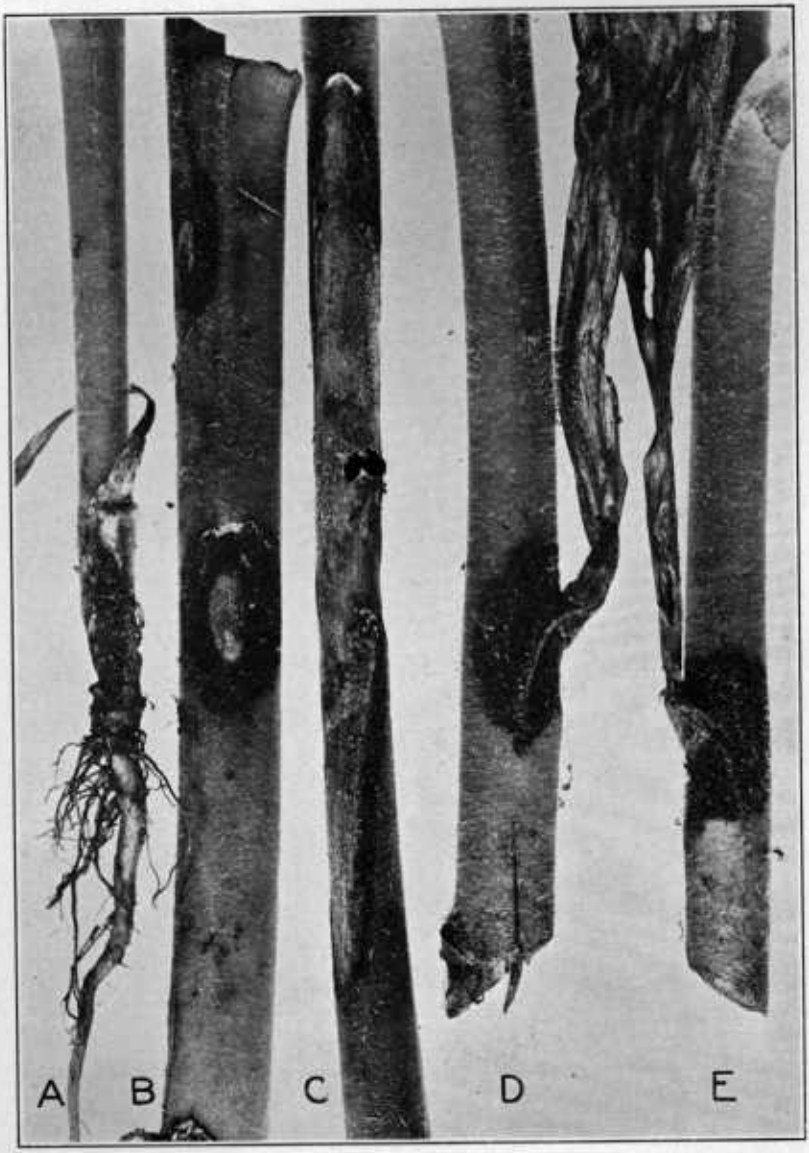

FIGURE 1.—Gray mold of tobacco. Plant A, girdled by fusion of several lesions originating at base of petioles. Plant B shows lesions produced at the nodes. Stem of C, girdled and a long lesion formed; portion distal to lesion dead. Two plants, D and E, show stem lesions originating from migration of Botrytis from leaf blade through petiole

is shown in Figure 5, A. Evidence of the splitting apart of cell membranes and of the pressure exerted by the hyphal tips as they force

apart the cells is presented in Figure 5, A and B. The vascular tissues of the petiole and stem are invaded in the same manner.

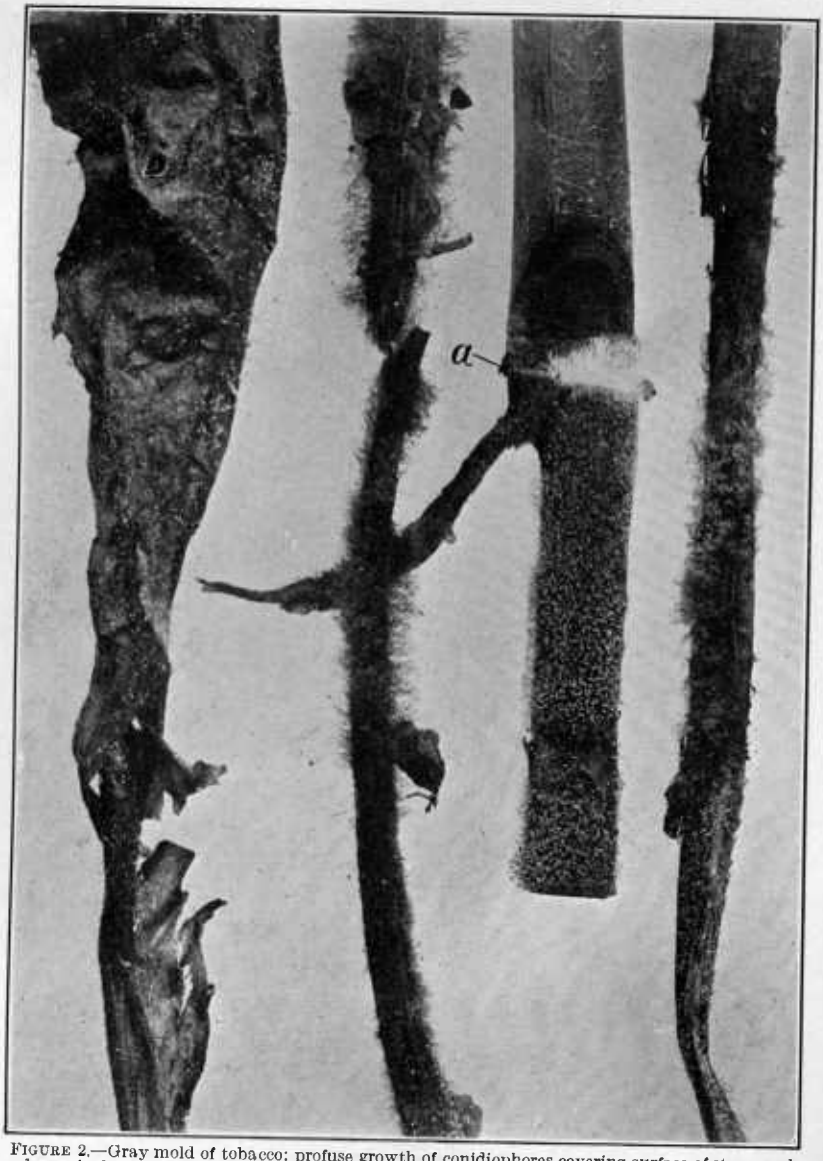

FIGURE 2.—Gray mold of tobacco; profuse growth of conidiophores covering surface of stems and leaves in damp weather. Note dense, white mycelial weft on surface of notch *a* cut in large stem at margin of lesion and coating of gray conidiophores on lesion. These growths resulted from placing the stems in a moist chamber for 24 hours

THE PATHOGENE

Gray mold of tobacco is caused by an organism that may be tentatively designated *Botrytis cinerea* Pers.[3] It is impossible to deter-

[3] Cultures and specimens were sent to Prof. H. H. Whetzel, Cornell University, Ithaca, N. Y., to whom grateful acknowledgment is made for this determination. Professor Whetzel from his study of Botrytis regards the tobacco gray-mold fungus as a member of the *cinerea* group.

mine from Persoon's description what fungus he had under observation, and therefore one can not be certain that the tobacco-seedling pathogene is specifically identical with the fungus described by him.

Some species of Botrytis are known only in the conidial stage, while others possess in addition an ascogenous and a sclerotial stage. The tobacco pathogene forms both conidia and sclerotia in culture. The hyphae are coarse and vacuolate, both in lesions and in culture. (Fig. 4, D.) Each conidiophore consists of a stout, erect branch with a few lateral branches on which botryose clusters of conidia are borne.

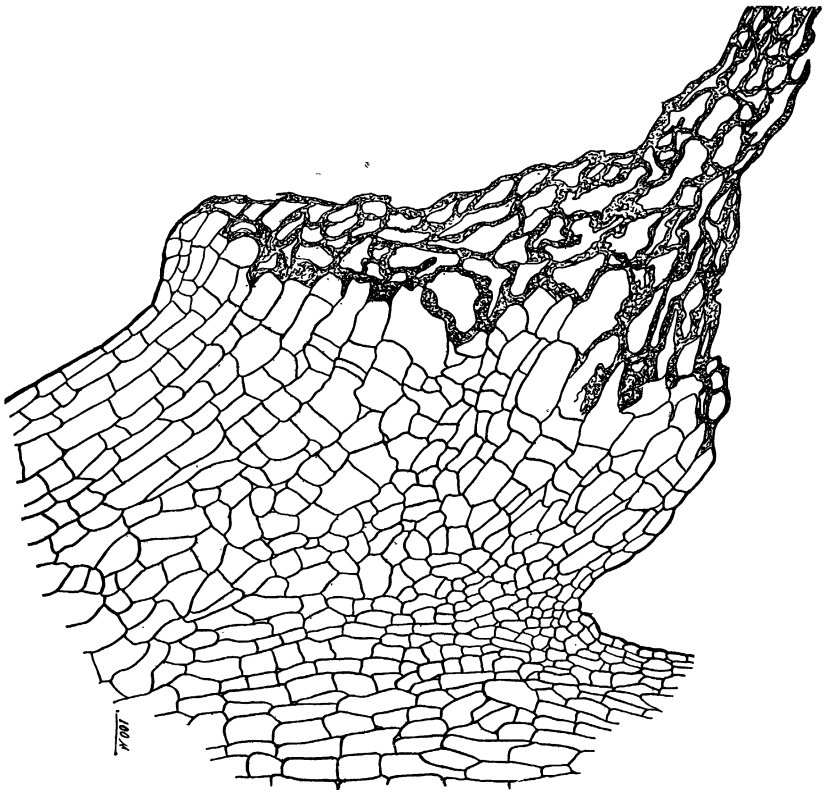

FIGURE 3.—Section of the petiole of a tobacco leaf in the region of its attachment to the stem and at one side of the center, showing the extension of a gray-mold lesion from the leaf blade through the petiole toward the stem. Those cortical tissues that have been invaded are in different stages of collapse

(Fig. 4, A.) The conidia are oval and are attached at the bluntly tapered end. (Fig. 4, B.)

A vigorous growth of grayish mycelium occurs on any of the ordinary culture media. Holdfasts arise at points where the mycelium comes in contact with the walls of the culture flask or test tube. (Fig. 4, E.)

The inoculation of tobacco seedlings with watery suspensions of conidia from pure culture resulted in infection only when a relatively high humidity was maintained. When inoculated plants were covered to conserve moisture they became infected, while those that

remained uncovered and from which the drops of suspension rather quickly evaporated remained free from infection. When *Botrytis cinerea,* isolated from decaying strawberries, was used as an inoculum, infections on tobacco resulted under the same conditions of inoculation. The inoculation experiments demonstrate that *B. cinerea* is pathogenic to tobacco only when conditions are made to approximate those in the seed bed. These conditions include the maintenance of films of moisture on the leaves for extended periods and the presence of leaves that are more or less weakened by age or by shading. This dependence on environmental factors accords with the writer's observations on diseases caused by Botrytis on lettuce, dewberries, and strawberries.

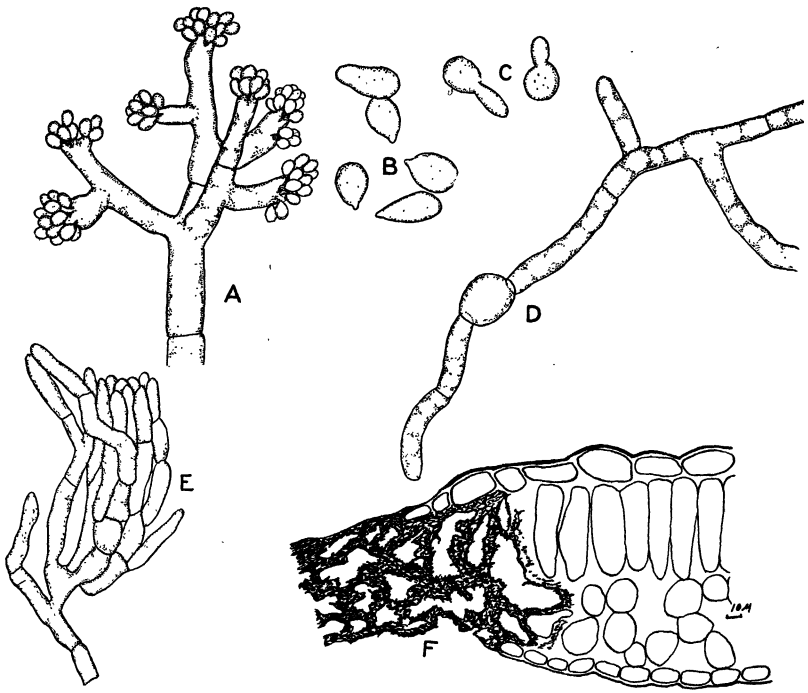

FIGURE 4.—A, Conidiophore of *Botrytis cinerea;* B, conidia; C, initiation of conidial germination; D, hyphal formation; E, a young holdfast; F, margin of a leaf lesion; all invaded portions are completely collapsed

The observations of Peters (*5*) on the occurrence of *Botrytis cinerea* as a parasite on tobacco seedlings and as a saprophyte on cured leaves and those of Pape (*4*) and of Böning (*3*) on its presence as a semiparasite on fading flowers indicate the possibility of the same species being concerned in the stem rot, poleburn, or house burn of tobacco investigated by Sturgis (*9, 10*) in Connecticut, Virginia, and Kentucky. Sturgis noted this decay on tobacco cut during warm, foggy weather and hung in curing barns, and identified the primary cause as *Botrytis longibrachiata* Oud.

Under the name "Dachbrand" or "Rippenfäule" Behrens (*2*) studied a decay of tobacco in curing barns in Germany, presumably identical with the stem rot described by Sturgis (*9*). In his opinion

the causal organism is *Botrytis cinerea*, of which *B. longibrachiata* is merely a strain or form. However, Sturgis's (*11*) further report of stem-rot studies maintains that *B. longibrachiata* and *B. cinerea* are easily separable because of differences in habit of growth, in color, and in size of conidia.

Whether the same species of Botrytis is responsible for the seedling disease in North Carolina and abroad, and whether it can also attack fading flowers and leaves that are curing, and, in addition, can produce a decay of cured leaves, can not be determined at this time. However, when the specific limits of *B. cinerea* are determined, the

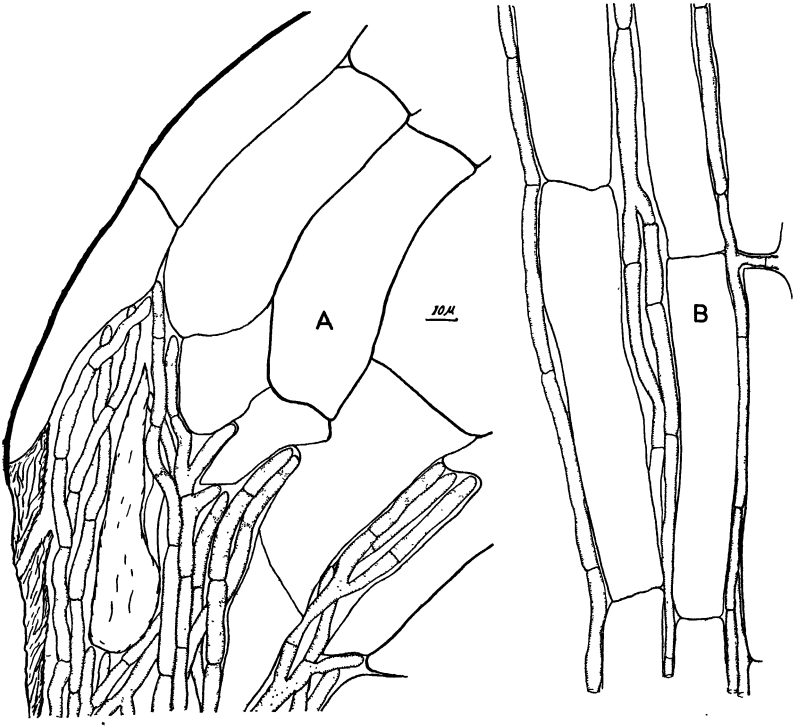

FIGURE 5.—A, Longitudinal section of a petiole showing the margin of a lesion. The hyphae of *Botrytis cinerea* are forcing apart the cells of the cortex after the dissolution of the middle lamella. B, Intercellular hyphae of Botrytis in the cortex of a stem lesion

possible identity of *B. vulgaris*, *B. longibrachiata*, and *B. cinerea* can be established.

INFLUENCE OF CLIMATIC CONDITION

The gray-mold fungus was found in nearly all of approximately 250 seed beds observed during the spring of 1928 and of 1929. In contrast with this condition, it was noted in only two of the 257 seed beds examined in 1930. An attempt was therefore made to correlate moisture and temperature conditions with the general prevalence of gray mold of tobacco during these two successive years and by this same procedure to account in large part for its almost entire absence during 1930.

It should be recalled in this connection that diseases caused by Botrytis are known to be favored by excessive rainfall and high relative humidity. Specific attention has been called to this fact by Stevens and Wilcox (*8*) in the case of a rot of strawberry fruits, both in the field and during transit. Moreover, studies on the temperature relations of the gray-mold fungus (*7*) show that it grows best at 25° C. (77° F.), that its minimum is near 0° C. (32° F.), and that it is rather sharply impeded at 30° C. (86° F.).

The meteorological data [4] for the period during which the tobacco seedlings were developing in the seed beds are assembled in Table 1.

TABLE 1.—*Precipitation during winter months of seasons 1927–28, 1928–29, and 1929–30, and precipitation and temperature during spring months of 1928, 1929, and 1930, at Oxford, N. C.*

Month and year	Precipitation	Mean temperature		Days with indicated temperature		
		Maximum	Minimum	Maximum of 68° to 85° F.	86° F. or above	34° F. or below
	Inches	° *F.*	° *F.*	*Number*	*Number*	*Number*
December, 1927	4.07					
January, 1928	1.21					
February, 1928	3.03					
Total, winter months	8.31					
March, 1928	2.95	62	38	10	0	1
April, 1928	6.93	68	43	17	0	4
May, 1928	2.50	76	53	24	4	0
Total, spring months	12.38			51	4	5
Total, 6 months	20.69					
December, 1928	.93					
January, 1929	1.48					
February, 1929	7.24					
Total, winter months	9.65					
March, 1929	5.38	66	43	12	2	5
April, 1929	4.30	74	48	20	4	1
May, 1929	4.54	79	54	26	5	0
Total, spring months	14.22			58	11	6
Total, 6 months	23.87					
December, 1929	1.97					
January, 1930	2.27					
February, 1930	1.70					
Total, winter months	5.94					
March, 1930	1.57	60	35	7	0	15
April, 1930	2.32	71	44	15	3	5
May, 1930	3.70	81	56	22	7	0
Total, spring months	7.59			44	10	20
Total, 6 months	13.53					

The influence of weather on this gray-mold disease may be better understood by considering the rainfall for the three preceding winter months in connection with the data assembled in Table 1. The total precipitation from December 1, 1927, to February 29, 1928, was 8.31 inches, which, with 12.38 inches for the spring months,

[4] Thanks are extended L. A. Denson, meteorologist, United States Weather Bureau, Raleigh, N. C., for making these data available.

makes a total of 20.69 inches for the 6-month period from December 1, 1927, to May 31, 1928. The total rainfall for the three winter months, December 1, 1928, to February 28, 1929, was 9.65 inches; for the three spring months, March 1, 1929, to May 31, 1929, 14.22 inches, making a total precipitation of 23.87 inches for the entire 6-month period. The total rainfall from December 1, 1929, to February 28, 1930, was 5.94 inches; for the three succeeding spring months, 7.59 inches, making a total for the half year of 13.53 inches.

The weather records for Oxford, N. C., do not cover a period sufficiently long to establish a normal, but the average normal for Henderson, N. C., and Durham, N. C., two near-by stations, is approximately 21.50 inches for the corresponding half year. The 1928 season, therefore, shows an approximate total deficiency of 0.81 inch; the 1929 season, an excess of 2.37 inches; and the 1930 season, a deficiency of 7.97 inches.

It may be noted from the tabulation that the precipitation for the three spring months of 1928, 1929, and 1930 was 12.38 inches, 14.22 inches, and 7.59 inches, respectively. The average normal for this period is 11.33 inches, making a departure from this normal of +1.05 inches, +2.89 inches, and −3.74 inches, respectively, for the three successive seasons. The season of 1930 was, in fact, so dry that the seedlings would have succumbed if growers had not watered the seed beds.

During the 92-day period while the seedlings were in the seed beds there were 51, 58, and 44 days in 1928, 1929, and 1930, respectively, during which the maximum temperature ranged from 68° to 85° F. for a portion of the day. This range may be regarded with fairness as optimum for the growth of the gray-mold organism. Unfavorable temperatures of 86° or above prevailed for 4, 11, and 10 days, and of 34° or below for 5, 6, and 20 days in 1928, 1929, and 1930, respectively, making a total of 9, 17, and 30 days on which unfavorable temperatures for the growth of Botrytis prevailed for a portion of the day. There were, therefore, approximately twice as many periods in 1930 as in 1929 during which growth was hindered by unfavorable temperature and more than three times as many in 1930 as in 1928.

Peters (*5*) points out that gray mold in Java appears during moist weather. Behrens (*1*) noted that it attacks tobacco seedlings in Germany when excessive humidity is maintained in the beds. The present observations on the influence of climatic conditions on gray mold, therefore, accord with previous observations on diseases induced by Botrytis on tobacco and other plants.

CONTROL

No definite experiments have been conducted to determine means of prevention and control of gray mold. However, observations show that the disease is not equally destructive in all seed beds. Those situated on low-lying ground which is poorly drained and which in consequence remains wet during seasons of normal rainfall may be seriously damaged. This condition is further aggravated if proper air drainage is prevented by a dense growth of woods surrounding the seed beds. In fact, all severe infestations that have been seen occurred in situations of this type. If the beds are located on porous soil on well-drained slopes in situations where the movement of air

currents is unimpeded, there is little probability of material damage by gray mold. The proper selection of a site for the seed beds, therefore, appears to afford a satisfactory means of prevention.

SUMMARY

Gray mold of tobacco in North Carolina is caused by a fungus tentatively identified as *Botrytis cinerea*. This disease is undoubtedly identical with diseases attributed to this and other species of Botrytis in Germany, Italy, and Java. There is a probability that the same organism may be associated with "stem rot" of tobacco in curing barns in Connecticut, Virginia, and Kentucky.

Gray mold is mainly a disease of seedlings in the seed bed. It first appears on the lower leaves that are predisposed to attack because they are past maturity and are etiolated. The pathogene passes from the leaf into the stem, where dark, sunken, necrotic lesions are formed. In extreme cases the stems are girdled and the plants succumb while in the seed bed or after being set in the field. Affected parts are covered with a grayish coating of conidiophores and conidia.

The epiphytotics of gray mold in 1928 and 1929 are correlated with abundant rainfall and a large proportion of days when the temperature was optimum for the growth of the pathogene. The scarcity of this disease in 1930 is correlated with the dry season, the relatively small number of days with optimum temperatures for the growth of Botrytis, and the relatively large number of days with temperatures that impeded the growth of the pathogene.

Seed beds located on well-drained slopes with good air drainage are least liable to be attacked by gray mold.

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