NOVEMBER, 1922 BULLETIN 246

WILDFIRE OF TOBACCO

FREDERICK A. WOLF

NORTH CAROLINA
AGRICULTURAL EXPERIMENT STATION

CONDUCTED JOINTLY BY THE STATE DEPARTMENT
OF AGRICULTURE AND THE NORTH CAROLINA STATE
COLLEGE OF AGRICULTURE AND ENGINEERING

RALEIGH

CONTENTS

SUMMARY

1. This investigation is concerned with a leafspot disease of tobacco called "wildfire," which was first definitely recognized in North Carolina in June, 1917. It has subsequently been found in twenty-six counties within the state. It is now known to occur also in Virginia, South Carolina, Georgia, Florida, Tennessee, Kentucky, Ohio, Wisconsin, Pennsylvania, Maryland, Massachusetts, Connecticut, Vermont, and in several districts in South Africa.

2. Wildfire is of the type of disease which appears to come suddenly and may cause the crop to be practically worthless. It produces characteristic spots on the leaves whose most constant and dependable character in all stages of development is the wide, yellowish border or halo. Seed pods are also affected.

3. Wildfire is a specific infection and the germ or bacterium which causes it has been named Bacterium tabacum. This has been proven by repeated isolation from diseased tissues and by infection of healthy tobacco with the germ in pure culture. Bacterium tabacum is not known to be actively parasitic on any other crop than tobacco.

4. The disease has its origin in the plant-bed. Such agencies as infected seed, contaminated plant-bed covers, infested soil, and man himself are responsible for the introduction of wildfire into the plant-beds. It is carried to the field at time of transplanting by the use of diseased seedlings.

5. Moisture is essential not only for infection, but for the dissemination of the disease. Rainy weather, especially when accompanied by wind, favors the rapid spread of wildfire and new infections do not appear in dry periods. Nutritional factors also influence the progress of wildfire.

6. The only practical method of control centers around the growing of healthy seedlings. If the seed-beds can be kept free from disease, the fields will be free from it also. Prevention of wildfire in the plant-bed depends primarily upon the use of (1) disease-free seed or seed which has been disinfected, (2) new plant-bed cloths or sterilized old ones, and (3) new plant-beds or thoroughly fired old ones. No satisfactory means of checking the disease in the field is known.

WILDFIRE OF TOBACCO

By Frederick A. Wolf

In 1917 the North Carolina Experiment Station began the investigation of a leafspot disease of tobacco (Nicotiana tabacum) which was clearly unlike any of those previously described. Because of the rapidity of its spread and its destructiveness, tobacco growers gave the name "wildfire" to this disease. Some of the results of this investigation of wildfire have previously been reported (11, 12, 13, 14, and 15), while others have been withheld, pending the completion of the work, or until such time as the publication of a more comprehensive account was warranted. Meanwhile, the disease has appeared in other states, where it has caused serious losses. Other investigators have published their results, which have confirmed in all essential points the findings of this station. They have contributed, furthermore, many additional valuable facts, some of which are in accord with our unpublished records. It is the present purpose, therefore, to bring together these data for the tobacco growers of North Carolina, since the essential features of this disease appear now to be well established, and since growers should be familiarized with the disease so as to recognize it and to intelligently apply measures for prevention and control.

HISTORY AND DISTRIBUTION OF WILDFIRE

In North Carolina

The disease was first definitely recognized in June, 1917, near Wendell, N. C., but it has been impossible to determine the number of years it existed prior to this date. Several reliable informants state that it caused the loss of practically the entire crop in one field in the same vicinity during the previous year. Another correspondent states that he is of the opinion that wildfire was present in 1916, near Apex, N. C. Both E. G. Moss, assistant director in charge of the Tobacco Branch station, Oxford, N. C., and the writer are convinced that the same disease was observed by them in fields near Oxford and Creedmoor, N. C., in 1916. As is the case with many diseases, it is impossible to actually prove the exact time and manner of introduction. Wildfire, doubtless, existed prior to 1916, but did not attract attention, since it is of the type of disease which appears in epidemic form only under certain environmental conditions as governed by rainfall, humidity, temperature, and nutrition. It has been impossible to satisfactorily evaluate these factors, but their influence will subsequently be discussed.

During the first season in which the disease was studied, specimens were collected in nineteen counties, namely: Alamance, Chatham, Cas-

well, Durham, Forsyth, Franklin, Granville, Guilford, Hoke, Johnston, Moore, Orange, Person, Rockingham, Stokes, Surry, Vance, Wake, and Warren. It has since been found in seven other counties: Beaufort, Craven, Davidson, Harnett, Lee, Martin, and Onslow. The area covered by these counties will be seen to include practically all of what is known as both the Old Belt and New Belt of North Carolina.

In Other States

Subsequent to the discovery of wildfire in this State, it has been found in other tobacco growing sections. Dr. James Johnson, United States Department of Agriculture, Office of Tobacco Investigations, and Dr. G. P. Clinton, Botanist, Connecticut Agricultural Experiment Station (1) observed the disease in Connecticut in 1919. During the next season it was observed in several localities in Massachusetts (2). In 1920 it was seriously destructive in Kentucky (10) and Tennessee. It has appeared in Virginia (4, 5) and, as shown by letters from plant pathologists and by the plant disease survey bulletins of the United States Department of Agriculture, occurs also in Georgia, South Carolina, Florida, Pennsylvania, Ohio, Maryland, Vermont, and Wisconsin.

In Other Countries

During 1921 the writer received a letter from Miss E. M. Doidge, Pretoria, South Africa, telling of the occurrence of wildfire in that section. Further, Clinton and McCormick (1, p. 520) have reported successful inoculation of tobacco plants in the greenhouse from wildfire specimens collected by Miss Doidge at Rustenburg and sent to him. The occurrence of wildfire in certain districts of South Africa, and the concern which it is causing, are also shown by statements in the journal of the Department of Agriculture (7), Union of South Africa, in March, 1921, as follows:

> A tobacco disease occurring in the Piet-Retief District and known to farmers as "Verterende roest" (literally, consuming rust) was brought to our notice by the chief of the Tobacco and Cotton Division. An officer of this division was detailed to investigate the matter. The disease starts on the lower leaves, which appear to be maturing prematurely, and spreads to the upper leaves. The disease was prevalent at the experiment station as well as on neighboring farms, and it was stated that only 10 per cent of the 1920 crop reached its normal development. The "verterende roest" is due to a bacterium, which is being carefully studied in the laboratory with a view of devising preventive measures which may be tested on a practical scale next season. Specimens of tobacco affected by a similar trouble have also been received from Rhodesia.

The April number of the same journal states:

> The bacterial disease of tobacco previously recorded from the Piet-Retief District is now spreading rapidly at Marikana in the Rustenburg District, and is probably very widespread. So far as the investigation has gone, it bears a strong resemblance to the wildfire of tobacco recorded in the United States.

In the January, 1922, number of the same journal, Evans (3) makes the following statement concerning the identity of the disease in South Africa:

> Tobacco wildfire (*Bacterium tabacum*), a serious disease, was investigated. It occurs extensively in the Pietersburg and Rustenburg districts. It was sent to us first from Rhodesia.

Wildfire is not known with certainty to occur in any other countries, but may be identical with a disease briefly described from the Philippine Islands by Reinking (8). This cannot be definitely determined, however, since several bacterial leafspots are now known to occur on tobacco.

ECONOMIC IMPORTANCE

It is impossible to give any satisfactory estimate of the losses caused by wildfire. During 1917 it was universally conceded by growers that this disease is the most destructive one which attacks tobacco. Losses, ranging from those which were inappreciable to those in which almost the entire crop was destroyed, were sustained in every locality where the disease was present. The problem of estimating the damage wrought is further complicated by the fact that in some sections the disease occurred on every farm, whereas in others it was present only in an occasional field. This lack of uniformity in destructiveness occurred also on the same farm, since certain fields were badly affected while others had little or none of the disease.

In 1918 wildfire was less prevalent than in 1917, and in none of the succeeding years has it been regarded as a trouble of major importance. In fact, it has appeared only in isolated areas and was confined to a few fields in any locality. It is interesting in this connection to note that no wildfire occurred during 1922 on the farms where the disease was first observed. The cause of this decrease in destructiveness and apparent disappearance of wildfire is not adequately known, but is a phenomenon which has been recorded in the case of a number of other plant diseases.

The investigations in several other states show a similar history of the occurrence and destructiveness of wildfire. Chapman and Anderson (2), for example, report that the disease appeared in 1920 in widely separated localities in Massachusetts, and in 1921 the infection was quite general throughout the state, with local centers of heavy infection and outlying situations free from disease.

The lack of uniformity in destructiveness is shown by Clinton and McCormick's (1, p. 394) inspection of 125 fields, of which 67 were infected. They state:

> Of the 67 fields, we can loosely classify them according to the amount of wildfire that showed at the time of the last examination, as follows: thirty-four with little injury, that is, less than 5 per cent; eighteen with a moderate amount; eight with much, and seven with very serious injury, in a few cases reaching almost a total loss.

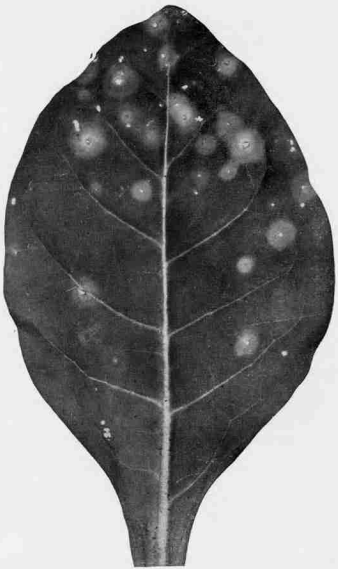

Fig. 1.—Young spots of wildfire on plant-bed leaf. The spots with dark centers, surrounded by a light border, characterize the second stage of the disease. The spots with white centers, surrounded by a halo, were formed around holes made by flea beetles. The white spots without halos were made by the feeding of flea beetles, with no resultant wildfire infections.

APPEARANCE OF THE DISEASE

Wildfire is essentially a leafspot disease, although it has been found upon the flowers and seedpods. It may appear upon the foliage in any stage of the plant from the time the seed leaves appear to maturity. The symptoms or signs of the malady upon the foliage are prominent, and differ from all other leafspots sufficiently to be easily recognized by the ordinary observer. Weather conditions and age of the affected leaf greatly modify the appearance of the disease.

In North Carolina wildfire makes its appearance in the plantbeds during the last week in April or the first week in May. Upon the smallest plants which have been dwarfed or retarded by crowding or have come from seed which were delayed in germinating, there appears what may be called a wet rot stage, which has not been described in the writer's previous accounts. The leaf margins and tips of such plants are involved in a rapidly advancing wet rot with a water-soaked zone between the living and the dead tissues. Often the entire leaf is rotted away, or the infected tissues may dry up and fall away, leaving the mutilated leaf apparently healthy. The bud-leaves of such plants are pale, erect, and slow in developing. Such plants may perish in the plant-bed or may not survive transplanting. This stage, which may properly be regarded as the first, involves plants in patches, and does not occur throughout the bed. The diseased areas are usually on the lower sides of the beds, in the dampest situations. This wet rot stage has frequently been observed in North Carolina in beds of which larger plants showed none of these symptoms. Chapman and Anderson (2, p. 69) and Clinton and McCormick (1, p. 381) have described this as a very early seedling stage, and it has been illustrated by the latter in their Plate XXIX, Fig. f.

On the larger plants, which have approached the size for transplanting, the first evidence of disease is the appearance of circular, yellowish green areas, about one-sixth to one-fifth inch in diameter. Within the next twenty-four hours, a minute, dead, brown speck, about the size of a pinhead, appears in the center of the spot, and the wide, yellowish green border, or halo, has become more prominent. This stage is followed within the next few days by an increase in size of both the central, dead area and the surrounding halo, forming a spot one-half inch or more in diameter, with a border of water-soaked appearance, which marks the margin of the central, dead, brown part. When the spots are numerous, they fuse, making large, irregular, dead areas. Not uncommonly, too, one-half of the leaf may be more seriously affected than the other, and in consequence, such leaves become twisted and distorted. This third stage is not prominent in the plant-bed, but shows to best advantage during June and July in the fields.

The width and prominence of the water-soaked band depends upon moisture conditions. In dry weather, the diseased areas are tan-colored to dark brown, with the lightest color at the center, and a narrow dark band at the border. During such weather these dead areas remain intact. When dewy nights and intermittent showers occur, however, the entire spot is soft and water-soaked in appearance, and the dead areas rot out so that the leaves present a ragged, torn appearance. This may properly be regarded as a fourth stage.

The most constant and dependable character for use in a field diagnosis of wildfire is the yellow halo, which persists in all stages of the disease. There is some yellowing with other leafspot diseases known to occur in North Carolina, such as angular leafspot, caused by Bacterium angulatum Fromme and Murray, frog-eye, caused by Cercospora nicotianæ E. and E., and Phyllosticta spot, caused by Phyllosticta nicotiana E. and E., but it is not prominent. One depends upon other characters, both macroscopic and microscopic, in distinguishing these several diseases.

Wildfire lesions on seed pods appear as rather prominent, brown spots. This form of the disease has been collected in several localities, but is not particularly characteristic, since other agencies cause the formation of quite similar spots on pods. The lesions on flower parts are small, brown and irregular, and have not been seen except in artificial infections.

CAUSE OF WILDFIRE

When the investigation of the cause of wildfire was first undertaken, it was found, just as is the case with many plant diseases, that various popular ideas were entertained as to the cause. Some growers believed that it was caused by improper fertilization, by rainy weather, or by certain insects. These several factors are now known to influence the progress and spread of wildfire, as will be discussed later, but unless the specific germ or organism is present, the disease will not appear.

It has been repeatedly demonstrated by carefully conducted experiments that this is a bacterial disease. The causal organism which bears the technical name of Bacterium tabacum, has repeatedly been isolated from diseased leaves and used in the successful inoculation of healthy plants. Our experiments on this point have been confirmed wherever the disease has been investigated, as shown by the results of Chapman and Anderson (2, p. 69), who state:

> In Massachusetts, the writers have made numerous isolations from all types of lesions described above, and have invariably obtained pure cultures of an organism which gave the same cultural tests as described by Wolf and Foster. (11). The same organism has never failed to produce the typical disease when healthy plants were inoculated with it from pure cultures.

LABORATORY STUDIES OF BACTERIUM TABACUM

In a previous publication (11) a rather full account of the morphological and cultural studies of this organism has been given. The writer's studies made subsequent to this publication, and the investigations of others show that this previous account (11) is in error in several important features. These features include size of the organism, number of flagella, ærobism, fermentation of carbohydrates and thermal death point. For this reason, a brief discussion of these discrepancies is desirable at this point.

Fig. 2.—Tobacco leaf, four days after inoculation with pure culture of Bacterium tabacum.

According to our previous account (11, p. 454), the size of the organism varies from 2.4 to 5 by 0.9 to 1.5 microns, the most common size

being 3.3 by 1.2 microns. A culture was sent to Dr. Fromme for examination, and he reported by letter, dated June 19, 1919:

> This organism measures 2.7 by 9.8 microns. We have used it for successful inoculations on tobacco, and have produced the type of spot which we have found both this year and last in seed-beds, and have considered it to be wildfire.

Slagg's (9) measurements of the germ isolated from Kentucky and Connecticut, and Clinton and McCormick's (1, p. 412) from Connecticut, are in agreement in showing that the organism varies from 1.4 to 2.8 by 0.5 to 0.75 microns. The explanation given by Clinton and McCormick (1, p. 425) to the effect that the large measurements may have been obtained from individuals which were undergoing division, accounts in part for the discrepancies. Further, one would not expect, as is well known, the measurements of different investigators to accord unless the same kind of stains were used.

By the use of Morrey's method of staining the writer was able to demonstrate a single polar flagellum. Slagg (9) states that the strains which he isolated possessed from three to six polar flagella. Clinton and McCormick (1, p. 412), with Moore's modification of Læffler's stain, demonstrated from one to four polar flagella in the following proportion, as determined by counts of several hundred: 40 per cent had a single flagellum, 45 per cent had two flagella, 13 per cent had three, and 2 per cent had four.

The wildfire organism is strictly ærobic, and does not cause clouding in the closed arm of fermentation tubes containing dextrose and saccharose, as previously stated (11, p. 455). This misinterpretation of the oxygen relation resulted from agitation consequent to handling the fermentation tubes (15a, p. 11) before the test had been concluded. Neither does it form acid from glycerin and lactose. This error arose from faulty preparation of these carbon compounds and can be avoided by methods discussed in another paper (15a, p. 9) and (15c, p. 45). These methods include a more accurate adjustment of the initial reaction of the media and avoidance of hydrolysis of the sugars by sterilizing in distilled water.

The thermal death point has been found to vary from 46°C to 51°C, depending upon the H-ion concentration (15c).

From the preceding consideration, it will be seen that the characteristics of wildfire, Bacterium tabacum, and angular leafspot, Bacterium angulatum, as tabulated by Fromme and Murray (6, p. 225) cannot be used to distinguish these two tobacco leafspot organisms. Observations on both diseases in the field show that they are clearly distinct, even though both occur upon the same plant. Inoculations in the greenhouse result in the formation of spots which are readily distinguishable. The two organisms, which have been repeatedly isolated by the writer during the past four seasons and grown in comparative

cultural studies, reveal certain constant differences which do not lend themselves readily to description. However, they may readily be separated by certain carbohydrate fermentations. The wildfire organism is able to ferment mannit and galactose with the formation of acid, neither of which compounds are attacked by the angular leafspot organism.

ISOLATION AND INOCULATION

Experiments.—Cultures of the wildfire organism from spots on tobacco leaves have been obtained by the use of either of two methods. Fragments of tissue from the margin of affected areas were washed in mercuric chlorid solution, then rinsed with sterile water, after which they were placed on poured plates of nutrient agar. Several types of colonies developed along the margins of these fragments. It was possible in some cases, by making transfers directly from these colonies, to secure pure cultures; while in others, dilution-poured plates were first made. Isolations were best effected by macerating diseased tissues in a drop of sterile water on a microscopic slide and then transferring a platinum loopful of this material to the edge of an agar plate. The inoculum was then spread with a zig-zag stroke toward the opposite edge. After two days incubation, numerous colonies had developed which were sufficiently isolated near the end of the stroke to permit fishing the wildfire organism to tubes of media.

Several unsuccessful attempts were made by the poured-plate method to isolate the germ from seeds which were suspected of being contaminated. These seeds were collected in late summer from diseased fields near Henderson, Oxford, Hillsboro, and Wendell, N. C., and in the following May were washed in sterile water and the washings were plated. Colonies of a considerable number of different bacteria developed, but none of them could be recognized as those of wildfire. It is known that wildfire can remain alive for several months on the surface of tobacco seed, since it has been isolated by the writer from artificially contaminated seed two months old.

Since it was believed that diseased leaves might serve as a means of over-wintering the disease, infected leaves were gathered in the fall near Louisburg, N. C., and allowed to remain in the laboratory until May. Attempts to isolate the germ from them were unsuccessful. Even though wildfire were known to be present, it would be exceedingly difficult to isolate it from such material.

Furthermore, three samples of cured leaves were tested in efforts to determine whether wildfire can survive the process of curing. As would be suspected, the germs cannot survive, for several hours, temperatures of 180°F and above, as are maintained in the last part of the curing process.

Clinton and McCormick (1, p. 411) secured cultures from an old dried leaf which had been kept in the laboratory nine months, but were unsuccessful in all other attempts to isolate from dried leaves and seeds.

FIG. 3.—Artificial infection with wildfire isolated from flea beetles.

It has not been possible to isolate wildfire from cloths taken from plant-beds, even though convincing proof, as will be indicated later, has been adduced to show that cloths can harbor the germs.

Flea beetles from diseased plant-beds were collected in six sterile test tubes on April 24, 1919. The beetles were captured by permitting them to jump into the opened tubes, which were placed near them, but were not brought into contact with the affected leaves. A few drops of sterile water were then poured into the tubes and the washings were

used in making poured plates. Numerous colonies of wildfire appeared on these plates. Isolations were made from these plates and their identity proved by the successful artificial inoculation on tobacco. The transmission of wildfire by flea beetles is a fact supported indirectly by the frequency with which the disease appears around the holes which they have eaten through the leaves. Observations in Connecticut (1, p. 381) are in accord in showing the origin of the disease at perforations made by flea beetles.

In the inoculation experiments, which were performed to determine the parasitism of Bacterium tabacum, as reported previously (11) the inoculum consisted of either macerated affected tissues, or pure cultures, and it was applied to healthy plants by sprinkling or with an atomizer. In some instances the inoculated plants were covered with a bell jar or shaded with papers, and in others they were left exposed. In all cases, the first signs of infection were evident three to five days after inoculation, and characteristic spots had developed within a few days afterward. Seedlings, young plants, and fully grown plants, have proven equally susceptible. When the leaves have begun to ripen, however, the color contrast between the yellow border of diseased spots and the normal tissue is not as sharp as in younger leaves.

Attempts have also been made by sprinkling the washings from supposedly infected seed, from diseased leaves about nine months old, which had been soaked for several hours, and from cloths from diseased plant-beds to secure infections, but with negative results in all cases. It appears quite probable, in the light of the experiments of Clinton and McCormick (1, p. 417), that infections might have resulted had the leaves been punctured. They succeeded in infecting needle-punctured leaves with an inoculum made from leaves dried from 198 to 298 days. By the same method they found that the organism could over-winter in the field in badly disintegrated leaves and to a limited extent in the soil (1, p. 419).

On June 14, 1919, three tobacco flower clusters were atomized with Bacterium tabacum which had recently been isolated from seedlings in plant-beds. Five days later, the corollas, calices and leaves immediately beneath the flower clusters were profusely spotted. The affected tissues were found to be filled with bacteria, which were reisolated, and subsequently found to be those of wildfire.

INFECTION OF OTHER HOSTS

Early in the investigation attention was directed to the question of whether the weeds and cultivated plants closely related to tobacco might serve as hosts for the wildfire organism and thus be a source of infection, and in this way account for the over-wintering and spread of the disease. While examining a badly diseased tobacco field near Wendell, N. C., on July 20, 1918, small, yellowish spots, with pinpoint-like dead centers, were noted on cowpeas (Vigna sinensis) planted between the hills of

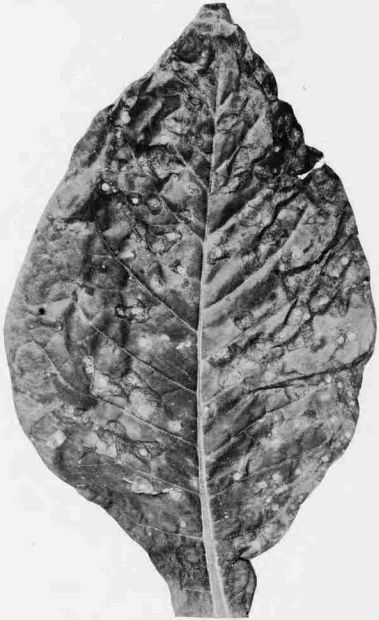

Fig. 4.—Mature wildfire spots, natural infection, on mature leaf. This stage shows spots with light centers and dark borders of water-soaked appearance.

tobacco. Isolations from these spots were used to inoculate 12 tobacco plants, with the result that typical wildfire developed. The germ was re-isolated from these spots on tobacco, and, together with the original cultures from cowpeas, were used in inoculating cowpeas. The bacterial suspensions were applied by sprinkling, but only a few spots developed upon the several plants employed in two sets of inoculations. These spots presented the same appearance as those on cowpeas growing in the field among the diseased tobacco.

The lesions, both naturally and artificially produced, are believed to have originated around punctures made by leaf-hoppers, which were abundantly present on these plants throughout the season. The wildfire organism is capable of multiplying within the cells weakened as a result of the withdrawal of their contents by the feeding of these insects, but is not able to parasitize normal cells. Drops of moisture laden with bacteria certainly dripped from the diseased tobacco plants to the cowpeas beneath them, and could thus have supplied the inoculum which caused the cowpea foliage to become spotted. This explanation is supported by the observation that the lesions on cowpeas did not increase in size beyond pinpoint-like dead areas, indicating that Bacterium tabacum cannot adapt itself to invade healthy tissues, and by the further fact that no new spots developed subsequently on the naturally and artificially inoculated plants. These experiments and observations are believed to show that the wildfire organism is not parasitic upon cowpeas, since strict or true parasitism involves the ability of an organism both to effect its own entrance, thus not necessitating entrance through wounds, and to invade healthy cells. It is certainly not actively parasitic, although there are those who might interpret these data as showing that it is semi-parasitic.

Among the plants closely related to tobacco which were inoculated, without securing infections, were Irish potatoes (Solanum tuberosum), tomatoes (Lycopersicum esculentum), peppers (Capsicum annuum), egg-plant (Solanum melongena), jimson weed (Datura tatula) and horse nettle (Solanum carolinense). Chapman and Anderson (2, p. 74) found lesions on tomato plants growing among tobacco in an infected plant-bed. These lesions appeared to have started around injuries of some kind, and from them they isolated cultures which developed typical wildfire lesions on tobacco. Furthermore, lesions developed around punctures on inoculated egg-plants and pokeweed (Phytolacca decandra). Successful inoculations occurred on petunia when no punctures were made. Similar results are reported by Clinton and McCormick (1, p. 420), who needle-punctured the leaves of young plants of tomato, pepper, egg-plant, jimson weed and pokeweed. All failed of inoculation except possibly at a few punctured places on pepper and egg-plant, on which faint yellowish spots appeared.

It is apparent from these experiments that up to the present the wildfire organism is not known to be actively parasitic on any other species except tobacco.

ORIGIN OF WILDFIRE

1. In the Plant-bed:

Since the disease was first seen by the writer on young transplants and the beds from which these plants were taken were found to be infected, efforts were directed toward a determination of the source of infection in plant-beds. Observations in North Carolina, extending through several seasons, show that wildfire invariably begins in the plant-bed and is introduced into the field at time of transplanting. The observations of others in Virginia, Massachusetts and Connecticut, confirm this point. As is apparent, the development of rational methods of control centers around a knowledge of the source of initial infections. At least four possible sources, diseased seed, infested soil, contaminated plant-bed covers, and man himself, have thus far come to light to account for the origin of the disease in the beds. Evidence relative to several other sources of infection, which have proven to be improbable, has been secured and will also be briefly discussed.

(a) *Seed.* The best evidence which has yet been secured that wildfire is seed-borne comes from the finding that the seed-pods are subject to attack, thus making entirely possible the contamination of the seed within the pods. It seems reasonable to suppose, too, that seed might become contaminated during harvesting and cleaning, if the foliage of the seed plants were diseased. Furthermore, seed which were artificially contaminated in January from pure culture, were sown about the middle of February in experimental plant-beds at Oxford, N. C. These beds were separated into compartments 8x6 feet in area with the board partitions extending into the soil for several inches. When the beds were examined, on May 7, all the plants in the two compartments in which contaminated seed were sown were seriously infected with wildfire. The spread of the disease to other compartments was checked by heavily sprinkling these beds with a strong solution of formaldehyde. No evidences of the disease could be found in any of the adjoining compartments, and none developed subsequently.

Clinton and McCormick (1, p. 372) are of the opinion that seed is probably one of the sources of infection, and cite the case of a grower who, in 1920, saved seed from a field in which the crop was diseased. Wildfire appeared in his plant-bed in 1921, and also in the beds of three other growers to whom he had given some of this seed.

(b) *Infested soil.* Because the isolation of plant pathogenic bacteria from soil is such an extremely difficult task, it has been impossible to absolutely prove that soil from fields or plant-beds which have grown diseased plants, is a source of wildfire infection. The evidence which has been secured along this line, however, indicates that account must be taken of soil as a source of infection, especially in plant-beds. In the experiments on soil infestation, old plant-beds were used which had borne diseased plants during the previous year, and which were not fired prior to planting. Seed from a locality where wildfire was absent were sown in these beds and new cloth was used as covers. The disease developed in several of these beds, but not in all.

In another test, seed were planted in flats in the greenhouse in soil from a field near Zebulon, N. C., in which the crop was practically a total loss. The seedlings in these flats showed no evidence of wildfire, due, perhaps, to the lack of suitable moisture conditions. The attempts to prove the presence of the organism in this soil by sprinkling a suspension on healthy plants were also unsuccessful. Clinton and McCormick (1, pp. 376 and 419), however, succeeded in one trial in infecting tobacco plants with infested soil. One point of difference between these experiments may account for the writer's failure to secure infection, namely: their plants were needle-punctured at time of inoculation, whereas, no injury was inflicted in our experiments.

(c) *Contaminated cloths.* No positive proof has been secured that cloths from infested plant-beds are a source of infection to beds in the succeeding year. It is conceivable, though, that old cloths might harbor wildfire, when it is recalled that the cloths are not removed until a few days before time for transplanting, at which time they are usually rolled up and put under shelter. As has been stated on another page, it has been impossible to isolate wildfire from used cloths, but indirect proof that such cloths harbor the parasite is shown by the following experiments. Near Henderson, N. C., new plant-beds, thoroughly fired, and so located with reference to distance and to surface drainage that there was no chance of contamination from neighboring fields, have been used in these tests. When seed of known healthy origin were used in certain of these beds and the beds were covered with new cloths, the plants remained free from wildfire. When, however, other beds were planted with seed from the same source and covered with cloths taken from beds which had been affected with wildfire during the previous year, the disease appeared.

Fromme (5, p. 1) cites the following incidents to show infection from the use of old cloths in Virginia:

> The germs of angular leafspot and wildfire may be carried on old canvas and cause infection in the plant-bed. This was proved by experiments, and also by the following cases: R. H. Mantiply, of Amherst County, used seed which had been treated but did not boil the canvas. Tilden Gooch used some of the same seed and boiled his canvas. Wildfire and angular leafspot were both found in Mr. Mantiply's bed, but not a trace of either could be found in the bed of Mr. Gooch.

(d) *Man.* Several instances of the introduction of wildfire into plant-beds by man himself have come under observation. Growers, unaware of the infectious nature of the disease and not familiar with

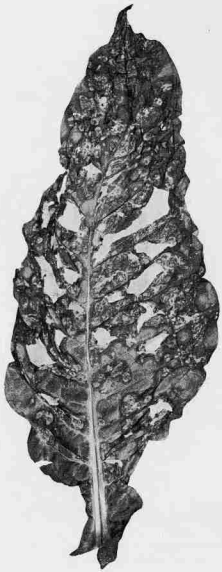

Fig. 5.—The rot stage of wildfire as occurs on mature leaves during rainy periods.

its appearance in plant-beds, on learning that it occurred in a neighbor's bed, trampled over the affected beds and indiscriminately handled the plants. They then examined their own beds and within a week infection appeared in their own plant-beds on the plants with which they came in contact. Instances have been noted, too, of the spread of wildfire from a few small infected areas over the entire bed through the operation of weeding.

(c) *Fertilizers.* Investigation of fertilizer as a source of infection was made since some growers contended that the introduction of wildfire into the plant-beds and the fields came through the fertilizer, and since tobacco stems were known to be incorporated in certain mixed fertilizers as a source of potash. A survey in several localities showed that wildfire occurred in plant-beds which had not been enriched by the use of commercial fertilizer, but in which manure had been used. Moreover, the disease occurred in some beds in which one particular brand of fertilizer was used but was absent in a neighboring bed in which the same brand was used. The possibility of the introduction of wildfire with diseased tobacco stems when they are incorporated with fertilizer material is excluded, since such stems are subjected to a sufficient degree of heat to insure their complete sterilization. There does not appear, therefore, to be any reason for believing that commercial fertilizers are in any way responsible for the introduction of wildfire either into the plant-beds or the fields.

2. In the Fields:

(a) *Seedlings.* During the several years in which this disease has been under investigation, numerous observations have been made on the origin of wildfire in the field. In every instance in which the disease occurred in the field, it was possible to find that the plants left in the beds after transplanting were also affected. Infected seedlings or transplants are the only source of field infections of any consequence. Chapman and Anderson's (2, p. 74) statement on this point is: "In all the field observations, we have seen nothing to indicate any other independent source of inoculum" (*i. e.,* than infected seedlings). Clinton and McCormick (1, p. 388) are also in accord with our findings, since they state:

> The first, and by far the most important, factor we need to consider in field infections is the seedlings used in setting out. Our experience last year indicated that if the grower can set his fields with plants absolutely free of wildfire, he has little to fear from this disease.

FACTORS INFLUENCING THE SEVERITY AND SPREAD OF WILDFIRE IN THE FIELD

Both nutritional and weather conditions have been observed to exert a controlling influence on the severity of wildfire once it is present in the field. Experienced growers have learned that the excessive use of nitrogen, especially nitrate of soda, produces a rapid, tender, watery

type of growth. The same type of growth results from proximity to ditches or to situations which are normally well supplied with water and organic matter. Furthermore, all observations are in accord in showing that wildfire is much more destructive to such plants than to those which have made a normal growth. It has also been learned that the use of an adequate supply of potash produces plants which are hardy and less liable to succumb to leafspot diseases. These observations certainly cannot be interpreted to mean that fertilizers or the several mineral elements cause wildfire, but only influence its progress. Unless the wildfire organism is present, the disease will not appear, regardless of the brand or kind of fertilizer.

Fig. 6.—Wildfire, natural infection, on tobacco seed pods and calyx lobes.

All observations, not only here, but in other states as well, are in accord in showing that moisture is of primary importance in influencing both the severity and spread of wildfire. Whenever a rainy period of several days' duration occurs, it may be followed by an outbreak of the disease. Few or no new infections occur during a dry period. This was strikingly shown during the summer of 1917, when two prolonged moist periods occurred, and in each case were followed by severe outbreaks with no appreciable spread in the interim.

If the rain is accompanied by high wind, ideal conditions are provided for the rapid spread of wildfire. A considerable number of cases recounted to the writer by growers are, in general, like the following observation. In one locality, near Eagle Rock, N. C., no disease occurred except in the case of a field of approximately an acre of newly-cleared land. The plants used in setting this field were unknowingly procured from a plant-bed which contained wildfire infected plants.

When this field was again observed, about a week after a rainstorm, which occurred about the middle of July, a field in the direction in which the wind blew was seriously diseased. The infection was much more abundant near the diseased field, and gradually diminished in severity in the direction away from the diseased field. It was very obvious here that the infection was spread by wind-blown rain. Wind, alone, however, during dry periods does not spread the disease.

The rainfall in North Carolina during the growing season of 1921 was generally considerably less than normal and wildfire was destructive only locally. During the past season, which has been excessively wet, little or no damage was occasioned by this disease. This is contrary to what was expected, but may have been occasioned by the drought of the preceding year.

YEARLY CYCLE OF THE WILDFIRE GERM

From what has been stated in the foregoing account, it is seen that the germ can over-winter on the surface of tobacco seed, in the soil of plant-beds, in old tobacco cloths or covers, and in decaying tobacco plants, and is introduced into the beds from these sources. The disease appears first on seedlings in the plant-bed, although plants of all ages are subject to attack. The germ enters the leaves through natural openings or through wounds. These natural openings include stomates or breathing pores, which occur on both leaf surfaces, and hydrathodes, or pores located at the leaf margins for the exudation of water. The bacteria float or swim into these openings in the film of moisture which covers the leaves when they are wet with dew or rain. Then after an incubation period of three to five days, the first signs of disease make their appearance.

Once inside the leaf, the bacteria multiply rapidly between the cells and cause their collapse and death, which is evidenced by the diseased spots. The tissues of these spots become filled with dense masses of bacteria, and when raindrops fall upon them the bacteria are splashed to other leaves of the same plant or to neighboring plants. Infected seedlings or transplants are the source of the introduction of the disease into the field at the time when the plants are set out. The progress of the disease in the field and its dissemination are governed primarily by moisture conditions. Some idea of the rate of growth of wildfire can be gained by the observation that under favorable conditions in culture it can reproduce itself every two hours so that countless millions are formed from a single organism within a few days. If certain of the diseased plants are left for seed, the pods become infected and the seed contaminated, and thus they harbor the germ until the next planting season. It is not impossible, too, that other means of survival during winter exist aside from those which have been investigated.

RECOMMENDATIONS FOR CONTROL

Plant-beds:

The fact of greatest significance which has thus far been established in regard to wildfire and which must be made the basis for all remedial measures is that the disease has its origin in the plant-bed. Once the disease has been established in the field, there is little that can be done to check its spread. The disease must be prevented from being introduced into the fields at time of transplanting by the use of healthy plants. This can be accomplished by the following procedure:

(1) Use seed of known healthy origin, or which are known to have come from a wildfire-free field, and which has been protected at all times from subsequent contamination. If such seed cannot be procured, then place the seed in a cheesecloth bag in a jar, or pour them into a jar and cover the top with a cheesecloth. The seed should then be soaked for ten minutes in a formaldehyde solution. This solution should be of the strength of one tablespoonful of formaldehyde to one pint of water. It is necessary to observe two precautions or injury to germination will result. The seed should not be treated over ten minutes and they should immediately be thoroughly washed in several changes of water. They are then ready to be spread out to dry. It is preferable to treat the seed a few hours before sowing. Good results, both in North Carolina and Virginia, have followed seed treatment.

(2) If wildfire was present in the beds the previous year, either make beds on new land away from all possible contamination by drainage from diseased beds or diseased fields, or thoroughly burn the old beds. This is necessary, since the infection is known to live over in the soil. If wildfire is absent and one wishes to use the same plant-bed for several years, it is well, as a precautionary measure, to pull up all plants left as soon as the transplanting season is over and to cover the bed deeply with pine straw, so as to guard against plant-bed diseases, to keep down all weed growth, and to conserve fertility.

(3) Use new plant-bed covers, or if old ones are employed, they should be sterilized by heating in boiling water. This precaution is necessary, since it has been found that old covers are a source of infection.

(4) Avoid infecting your own beds by not visiting the infected beds of neighbors.

(5) As a final precaution, the plant-beds should be sprayed or dusted with Bordeaux mixture. If commercial preparations are used, the package contains directions for use. If homemade Bordeaux mixture is employed, it should consist of one pound of bluestone dissolved in a gallon of water, and one pound of quick lime slaked in a gallon of water. The two should then be poured together and diluted with water to make ten gallons of spray. This quantity should be sufficient to cover about 600 square feet, if properly applied. Begin spraying about

four or five weeks before transplanting, and spray each week or ten days so as to keep the new growth protected with a coat of spray. Excellent control of wildfire in plant-beds has been accomplished in Connecticut and Massachusetts by spraying and dusting. No experiments have been conducted along this line in this state, but there appears to be no reason for doubting that spraying would be equally effective here.

Fields:

(1) Use only plants known to come from disease-free plant-beds in setting out the fields. Do not buy or exchange plants unless they are known to be free from wildfire. If it is impossible to get healthy plants and the beds are only slightly diseased, use only those which are

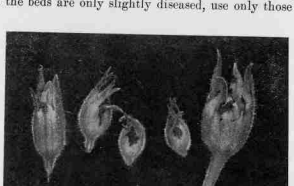

Fig. 7.—Lesions on young pods following inoculation from pure culture.

apparently free. Inspect the fields about a week afterward, remove the diseased plants and destroy them. Reset with healthy ones. After the plants have started to grow in the field, make a second inspection and remove the lower affected leaves. The elimination of infective material may effectively decrease the injury to the crop which might occur in late summer.

(2) Fields which have grown a diseased crop during the previous year should not be planted with tobacco. The disease is known to live over in the field and may infect the next crop when tobacco follows tobacco. If it is necessary for tobacco to follow tobacco upon infested fields, there appears to be little probability that the disease will become serious when healthy plants are set out. Fields in which the crop was badly diseased have been set with healthy plants in the following season and a healthy crop grown. It is well, however, aside from the danger of wildfire, to employ a system of crop rotation.

(3) Growers have frequently asked whether the disease in the field cannot be checked by spraying. The few experiments that have

been made along this line have not given very satisfactory results. Even if it could be accomplished, such a procedure cannot be recommended as a field practice, because of the expense involved and the possible injury to the market value of the crop.

(4) Field observations indicate that low-topping is to be avoided. Low-topped plants suffer greater injury by wildfire than plants with the normal number of leaves, especially when rainy weather occurs as the crop is maturing.

(5) The removal or priming of affected leaves is of doubtful value on a practically mature crop. In some cases it appears to have retarded the spread of wildfire, whereas, in others no good has been accomplished by priming.

LITERATURE CITED

(1) Clinton, G. P., and McCormick, Florence A. **Wildfire of Tobacco in Connecticut.** Conn. Agr. Exp. Sta. Bul. 239: 369-423, Pls. XXIX-XXXII, 1922.

(2) Chapman, G. H., and Anderson, P. J. **Tobacco Wildfire. Preliminary Report of Investigations.** Mass. Agr. Exp. Sta. Bul. 203: 67-81, Pl. 1, 1921.

(3) Evans, I. B. P. **Tobacco Wildfire (Bacterium tabacum).** Jour. Dept. Agr. Union S. Afr. 4: 57, 1922.

(4) Fromme, F. D. **Wildfire and Angular Spot.** Va. Agr. Exten. Div. Bul. 62: 25-31, 1920.

(5) Fromme, F. D. **Seed Treatment for Tobacco.** Va. Poly. Inst. Manifold copy 3250: 1-3, 1921.

(6) Fromme, F. D., and Murray, T. J. **Angular Leaf-spot of Tobacco, An Undescribed Bacterial Disease.** Jour. Agr. Res. 16: 219-228, Pls. 25-27, 1919.

(7) Klerck, G. W. (editor). **Departmental Activities, Botany.** Jour. Dept. Agr. Union S. Afr. 2: 210 and 310, 1921.

(8) Reinking, O. **Philippine Plant Diseases.** Phytopath. 9: 130, 1919.

(9) Slagg, C. M. **Preliminary Report on a Study of the Wildfire Disease of Tobacco.** Amer. Phytopath. Soc. Abstra., 1921: 25-26, 1921.

(10) Valleau, W. D. **Wildfire and Angular Leafspot of Tobacco.** Ky. Extension Div. Circ. 89: 3-16, Illustrated, 1921.

(11) Wolf, F. A., and Foster, A. C. **Tobacco Wildfire.** Jour. Agr. Res. 12: 449-458, Pls. 15-16, Figs. 2, 1918.

(12) Wolf, F. A., and Foster, A. C. **A Bacterial Leafspot of Tobacco.** Sci. N. S., 46: 361-362, 1917.

(13) Wolf, F. A. **Tobacco Wildfire.** N. C. Agr. Exten. Circ. 61: 1-4, Figs. 3, 1918.

(14) Wolf, F. A., and Moss, E. G. **Diseases of Flue-cured Tobacco.** Bul. N. C. Dept. Agr. 40: No. 12, 5-45, Figs. 24, 1919.

(15) **Studies on the Physiology of Some Plant Pathogenic Bacteria.** N. C. Agr. Exp. Sta. Tech. Bul. 20, p. 47, Fig. 1, 1921.

(a) II. Further studies on bacterial blight of soybean, pp. 9-13, Shunk, I. V., and Wolf, F. A.

(b) IV. Thermal death points of some bacterial plant pathogenes in relation to reaction of the medium, pp. 21-24, Wolf, F. A., and Foster, A. C.

(c) VI. The application of certain recent studies on technic to methods of culture of plant pathogenes, pp. 44-47, Wolf, F. A.