WATER SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE BLACKFIRE DISEASE OF TOBACCO[1]

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INTRODUCTION

In a previous publication [2] the general situation with respect to the wildfire and the blackfire or angular leaf spot diseases of tobacco (*Nicotiana tabacum* L.) was discussed and the need for explaining the occurrence of leaf spot epidemics was indicated. It was shown that epidemics of wildfire (*Bacterium tabacum* Wolf and Foster) are conditional on the occurrence of leaf water soaking, and the factors modifying leaf resistance to water soaking were discussed. These results are extended in the present paper by similar studies with the blackfire (*Bact. angulatum* Fromme and Murray) disease.

Blackfire is the common tobacco leaf spot disease of Virginia, North Carolina, Tennessee, Kentucky, and Wisconsin. Following its identification by Fromme and Murray,[3] it was long regarded as established that the disease was caused by *Bacterium angulatum* and was serious and destructive. It was a fact, however, that no one had ever produced the "epidemic" type of blackfire (see fig. 2, *A*) under controlled conditions. Inoculations produced only small localized lesions (fig. 1), and in consequence Valleau [4] suggested that the epidemic type of leaf spot was nonparasitic in nature. It is believed that the results presented in this paper adequately clear up this situation.

EXPERIMENTAL RESULTS

Methods of study and preliminary results with *Bacterium angulatum* paralleled in all details those given in a previous publication [5] for *Bact. tabacum*.

In repeated controlled experiments, it was demonstrated that the difference between disease development as shown in figure 1 and figure 2, *A*, was entirely dependent on leaf water soaking (fig. 3). Thus, for example, 12 leaves were selected and one-half of each was sprayed until a water-soaked condition of these areas was produced. Sixty-five of the water-soaked areas were then inoculated by pricking them lightly with a needle dipped in a suspension of the bacteria, and an equal number of inoculations were made on the unsprayed halves. The remaining water-soaked areas were left uninoculated. The inoculations of water-soaked areas produced lesions in every case, ranging

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[2] CLAYTON, E. E. WATER-SOAKING OF LEAVES IN RELATION TO DEVELOPMENT OF THE WILDFIRE DISEASE OF TOBACCO. Jour. Agr. Research 52: 239–269, illus. 1936.

[3] FROMME, F. D., and MURRAY, T. J. ANGULAR-LEAF SPOT OF TOBACCO, AN UNDESCRIBED BACTERIAL DISEASE. Jour. Agr. Research 16: 219–228, illus. 1919.

[4] VALLEAU, W. D. ARE BLACKFIRE AND ANGULAR LEAF SPOT OF TOBACCO IDENTICAL? (Abstract) Phytopathology 19: 93. 1929.

[5] CLAYTON, E. E. See footnote 2.

from ⅜ to 1⅞ inches in diameter. The inoculations without water soaking produced either no infection or at most a lesion less than one-eighth of an inch in diameter. The water-soaked condition of areas that were not inoculated disappeared without any leaf injury. Furthermore, the lesions on the water-soaked areas developed so rapidly that they were of large size when the plants were removed from the damp chambers (60 hours after inoculation).

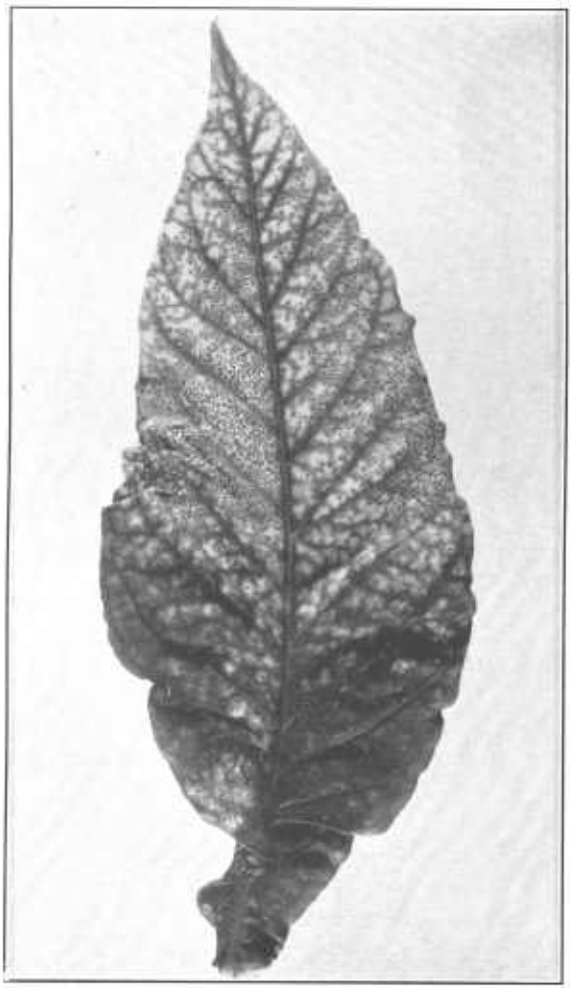

FIGURE 1.—Leaf inoculated by atomizing with a *Bacterium angulatum* suspension; photographed 20 days later. The lesions have attained full size and are typical of those obtained without the aid of water soaking.

INOCULATION OF UPPER AND LOWER LEAF SURFACE

In the work with wildfire it was found that, provided water soaking was present, there was little or no difference in the results from lower or upper leaf-surface inoculation. With *Bacterium angulatum*, which appears to be a less virulent parasite, there were significant differences in results between the two surfaces (table 1).

TABLE 1.—*Effects of different combinations of water-spray and inoculation treatments on the upper and lower leaf surfaces*

Leaf no.	Leaf surface given—		Initial lesions	Leaf area finally killed	Leaf no.	Leaf surface given—		Initial lesions	Leaf area finally killed
	Water spray	Inoculation				Water spray	Inoculation		
			Number	*Percent*				*Number*	*Percent*
1	Upper	Upper	36	20	1	Lower	Upper	125	20
2	do	do	36	50	2	do	do	13	10
3	do	do	20	20	3	do	do	70	10
1	do	Lower	250	80	1	do	Lower	224	40
2	do	do	154	50	2	do	do	168	20
3	do	do	150	100	3	do	do	450	50

Thus, with *Bacterium angulatum*, even with water soaking, the number of initial lesions and usually the subsequent disease damage were much greater from inoculation of lower leaf surfaces, though it did not appear to matter greatly whether the water soaking was produced by spraying upper or lower surfaces. The cheek inoculations of leaves not soaked that accompanied this experiment produced only scattering small lesions, and these caused practically no leaf damage.

EFFECT OF SIZE AND PERSISTENCE OF WATER-SOAKED AREAS ON LESION DEVELOPMENT

Experiments with size of water-soaked areas in relation to blackfire development showed that, as with wildfire, larger water-soaked areas

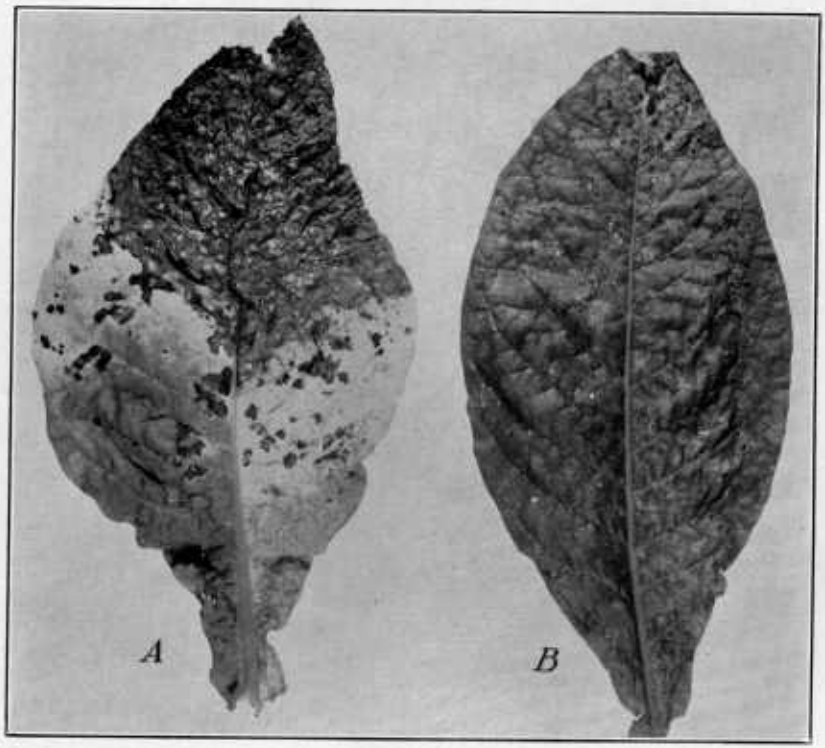

FIGURE 2.—The blackfire disease as it developed from artificial inoculation under favorable field conditions: *A*, Lower leaf, showing typical symptoms of the destructive epidemic type of disease which is usually called blackfire; *B*, upper leaf from the same plant, showing lesions of a type frequently described as angular leaf spot.

favored the development of larger lesions. Thus, the following results were obtained:

Leaves not water-soaked.—Twenty prick inoculations gave either no infection or a mere trace.

Leaves water-soaked, areas one-eighth to three-eights of an inch in diameter.—20 prick inoculations gave lesions ranging from one-eighth to three-eighths of an inch in diameter.

Leaves water-soaked, areas ¾ to 1½ inches in diameter.—Lesions ranged from one-fourth to three-fourths of an inch in diameter.

With small areas, the average size of the lesions was 0.3 inch, which was about the average size of the original water-soaked areas. The

lesions with largew ater-soaked areas averaged 0.7 inch in diameter, but this was much less than the size of the original water-soaked areas.

FIGURE 3.—Entire leaf inoculated with a *Bacterium angulatum* suspension. Right half previously water-sprayed; left half not water-sprayed.

Repeated tests have shown that this is the usual condition, namely, that small water-soaked areas are completely invaded but larger areas are not.

The time that the water-soaked leaf condition persists after infection has occurred is of great importance in blackfire development, as is shown in table 2 and figures 4 and 5.

The actual number of infections secured was about the same when the leaves were water-soaked for 10, 24, or 50 hours, but with the shorter periods development of the lesions was cut short and damage to the plant was slight. Figure 4, *A*, shows that it is quite possible for leaves to be freely infected by *Bacterium angulatum* and then, even after epidemic disease development is well under way, the progress of the disease can be abruptly checked. These results explain why even severe storms, when followed at once by clear weather, are not effective in producing blackfire outbreaks.

TABLE 2.—*Relation of persistence of water-soaked areas to disease development*

Leaf no.	Leaf area killed after 10 days when water soaking was continued for—			Leaf no.	Leaf area killed after 10 days when water soaking was continued for—		
	10 hours	24 hours	50 hours		10 hours	24 hours	50 hours
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1	1	3	20	8	2	3	20
2	1	8	15	9	1	5	75
3	1	5	15	10	2	10	60
4	1	5	25	11	2	8	75
5	2	3	25	12	2	8	75
6	1	5	25				
7	6	2	15	Average	1.8	5.4	37.1

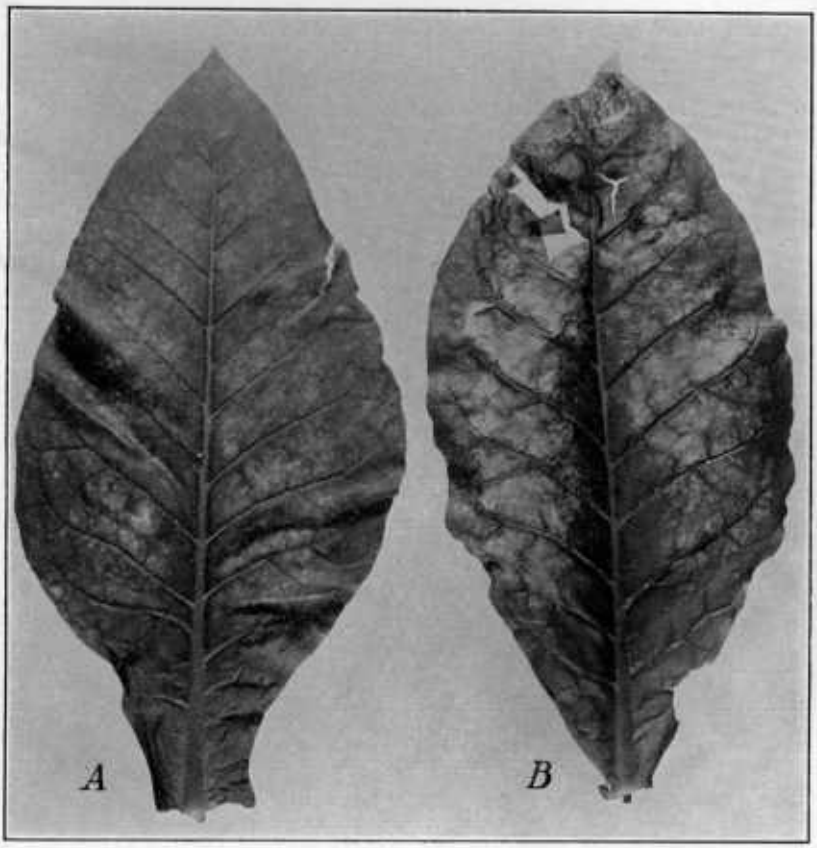

FIGURE 4.—Effect of duration of water-soaked condition on disease development. Both leaves were water-soaked and inoculated alike. With *A* the water-soaked condition was maintained in a saturated atmosphere for 24 hours; with *B*, for 50 hours. Note the heavy infection but arrested disease development on *A;* with *B*, the final result was extensive tissue destruction.

EFFECT OF TOPPING AND FERTILIZATION PRACTICES ON SUSCEPTIBILITY TO BLACKFIRE

Height of topping has a marked effect on the susceptibility of the host plant to epidemic blackfire, just as was previously shown for wildfire. In table 3 results are given from a field test conducted in 1935. All plots were uniformly inoculated and conditions were moderately favorable for disease development. Low-topped plants averaged 10 leaves per plant; high-topped plants, 18 leaves; plants not topped, 25 leaves. The percentage of leaf area destroyed by disease was estimated for 40 leaves from each plot.

Topping effects are at once marked and consistent, and low topping is undoubtedly a major factor in promoting blackfire damage in the dark-fired tobacco-producing areas where this practice is essential to produce a large, heavy type of leaf.

It was not possible to make so detailed a study of fertilizer influences on blackfire development as was made on wildfire development. However, numerous observations indicate clearly that low-potash and high-nitrogen fertilization increase blackfire damage. These fertilizer effects were very apparent in plot work conducted on soils of

low natural fertility, where the same fertilization treatments were repeated year after year; but on moderately fertile soils, with the application made but a single year, even marked variations in the quantities of nitrogen and potash applied in the fertilizer had but little effect on blackfire development.

FIGURE 5.—Effect of water soaking and duration of water-soaked condition on blackfire development for entire plants. Both plants were water-soaked and inoculated alike. *A* was then held in a saturated atmosphere for 10 hours; *B*, for 50 hours. Photographed 12 days later.

TABLE 3.—*Percentages of leaf area killed following different heights of topping*

Low topping[1]	High topping[1]	No topping[1]
60-30- 8-20-15	2- 5-30- 5-30	8- 2- 5- 2- 2
5-12-20-15- 5	10-10-10- 6-10	5- 1- 5- 1- 4
2- 4- 6-10-20	2- 5- 3-15- 5	4- 1- 2- 2- 3
10-12- 4-50-10	10- 5- 4- 8- 6	.5-. 5- 2-. 5- 2
30-10-20- 8-30	8- 3-10-10-10	3- 5- 2- 8-25
25-15- 5-10- 2	5- 8- 8- 2- 8	4- 5-20-40- 1
20-12- 5-35-30	8-40- 2- 8- 8	2- 5- 3- 4- 5
20-25-20-30- 2	5-30- 8- 4-15	.5- 1- 2- 2-25
Average 16.8	Average 9.5	Average 5.4

[1] Each of the following 40 percentages refers to a separate leaf. The values are arranged in groups of 5 merely for convenience and to save space. No relationship exists among values occupying similar positions in different columns.

DISCUSSION

The results secured in these experiments with blackfire, caused by *Bacterium angulatum*, are similar in every respect to those previously reported for wildfire, and it seems clear that with types of tobacco grown in the United States epidemic development of blackfire is dependent on water soaking of the leaves. Water soaking breaks down host resistance and permits successful and rapid tissue invasion by the organism.

Bacterium angulatum, while similar in mode of action to *Bact. tabacum*, is evidently a less virulent parasite. Thus, throughout the work

in parallel experiments, infection with *Bact. angulatum* in the absence of water soaking was less easy to secure and the infections were less numerous, developed more slowly, and were smaller; with water soaking and more favorable conditions, these differences still persisted though they were not so marked. With favorable conditions, both organisms can practically destroy a crop in a short time.

Some degree of blackfire protection can be secured by high topping, and low-nitrogen and high-potash fertilization is also helpful, but these practices can be applied only in areas where the type of tobacco grown will permit. They are applicable to the flue-cured area.

However, it is evident that effective blackfire control must be sought by other means, and the most promising of these are (1) sanitation and other measures designed to eliminate sources of infection and (2) development of varieties still more resistant to the disease than those now available. In the latter connection it is to be noted that varieties grown in the United States are practically all moderately resistant to blackfire, which accounts for the fact that they suffer little damage from the disease until this resistance is broken down by the conditions incident to heavy storms.

SUMMARY

Tobacco leaves are readily infected by *Bacterium angulatum*, but under ordinary conditions invasion is limited to small areas. The lesions are usually one-eighth of an inch or less in diameter, and large numbers of infections cause but little damage to most types of tobacco.

It had been suggested that the large, quickly developing lesions characteristic of epidemic blackfire were nonparasitic in nature; but it is now shown that they are caused by *Bact. angulatum*, but only under special conditions. The resistance of the leaf to invasion must first be broken down by water soaking, which in turn results from severe storms.

Even after leaves are water soaked, however, and infection has occurred, the development of the disease is abruptly checked if the water-soaked condition disappears within a few hours. Epidemic disease development was repeatedly obtained by water soaking the leaves for 48 hours. Resistance of the leaves to water soaking, and hence to the disease, has been shown to be greatly modified by topping and fertilization practices. High topping and low-nitrogen and high-potash fertilization increase leaf resistance to water soaking, and these measures are recommended as practicable in the flue-cured tobacco area.

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