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TOBACCO SUBSTATION AT WINDSOR

REPORT FOR 1942

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Connecticut
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New Haven

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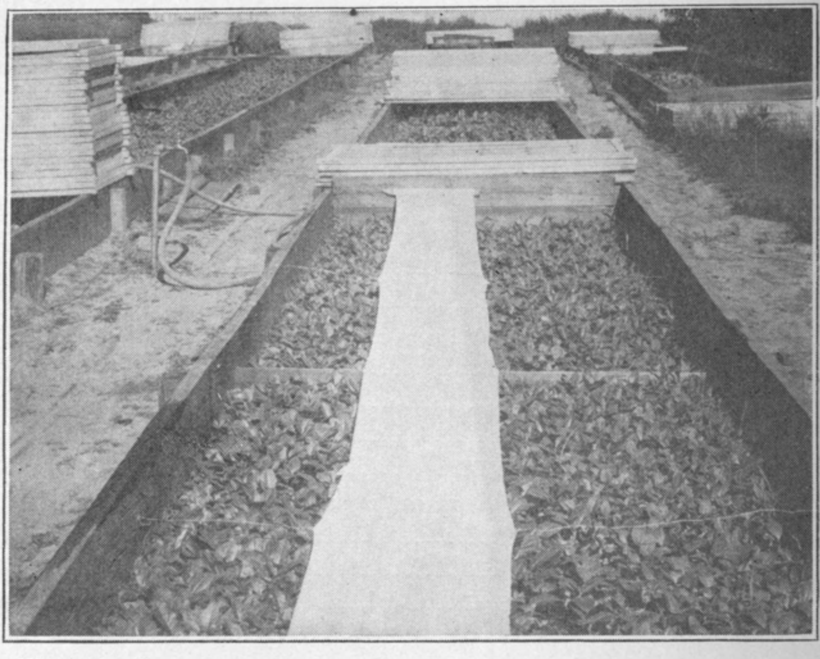

Figure 1. Control of mildew by paradichlorobenzene. The crystals are distributed on the long strip of cheesecloth at night.

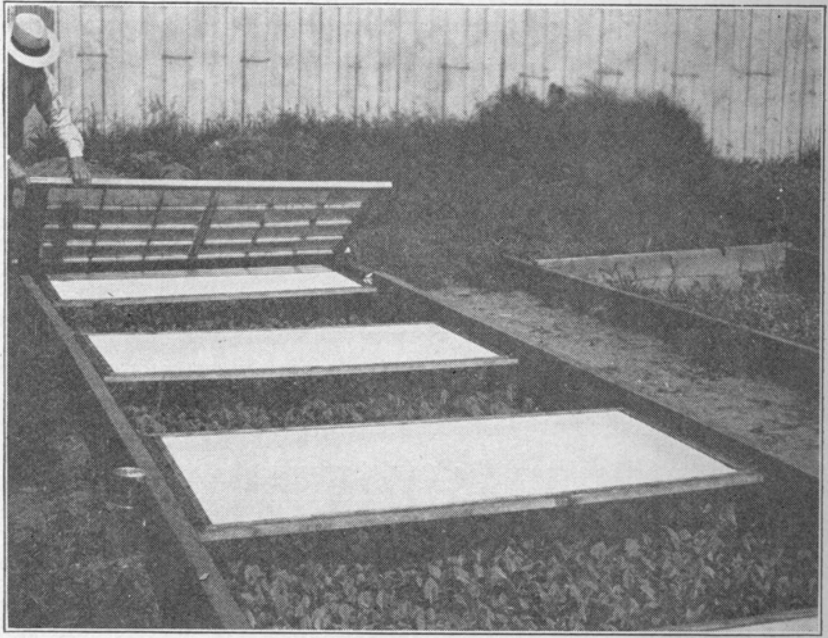

Figure 2. Another method of controlling mildew with paradichlorobenzene. Crystals are distributed on cloth frames just under the glass sash.

Tobacco Substation at Windsor

REPORT FOR 1942

P. J. ANDERSON, T. R. SWANBACK AND S. B. LECOMPTE, JR.

THIS, the Twenty-First Annual Report of the Tobacco Substation at Windsor, describes the progress of investigations during 1942. War scarcities of labor and materials, gasoline and tires, have changed to some extent the nature of the work and direction of emphasis, but progress has been satisfactory nevertheless.

TABLE 1. ACREAGE AND PRODUCTION OF TOBACCO IN THE CONNECTICUT VALLEY FOR 1942 AND 1941, AND A TEN-YEAR AVERAGE.[1]

	Acreage			Production in pounds		
Type	Average 1930–39	1941	1942	Average 1930–39	1941	(estimated) 1942
Broadleaf	8,660	8,000	7,500	13,341,000	12,808,000	11,931,000
Havana Seed	7,690	7,700	7,100	11,753,000	13,426,000	12,416,000
Shade	6,170	6,800	6,100	6,025,000	6,437,000	5,676,000
Total	22,520	22,500	20,700	31,119,000	32,671,000	30,023,000

[1] New England Crop Reporting Service release of December 29, 1942.

For growers, it has been a year of difficulties in finding adequate labor and supplies of fertilizers, fungicides and insecticides. The result has been a somewhat reduced acreage as indicated in Table 1, which gives comparisons with last year and a ten-year average of the acreage and production for the three leading Connecticut tobaccos. Reduction in acreage and increase in cigar consumption were responsible, however, for a somewhat better price paid for the crop.

Weather conditions during the growing season resulted in an average crop. Above-average rainfall in some sections caused the crop to be light in weight. Periods of continuously wet weather during the curing season resulted in some loss from pole rot. There were scattered hail storms, but in most cases they caused only a small reduction in crop value. Precipitation records, presented in Table 2, show that the rainfall was above the average for every month of the season.

In 1936, the U. S. Department of Agriculture assigned an entomologist, A. W. Morrill, Jr., to aid in the investigations on tobacco insects. His work on methods for the control of flea beetles was particularly outstanding and saved growers thousands of dollars. Other insects investigated were wire worms and thrips. His service as a consultant on questions of control of all sorts of insects was much appreciated by growers. Due to reduction in funds the bureau has found it necessary to transfer Mr. Morrill elsewhere. However, the work, especially on wire worms, will be continued as soon as a qualified man can be found.

Because it was necessary to reduce car mileage, the staff has not made so many farm visits, nor have so many growers driven to the Station for personal consultations. On the other hand, we do not find that there has been any considerable decrease in number of soil samples submitted for analysis or of seed samples for testing.

TABLE 2. DISTRIBUTION OF RAINFALL IN INCHES AT THE TOBACCO SUBSTATION, WINDSOR, 1942

		By ten-day periods	By months	Average for preceding 20 years
May	1–10	1.54	4.41	3.39
	11–20	1.77		
	21–31	1.10		
June	1–10	1.50	4.28	3.91
	11–20	1.38		
	21–30	1.40		
July	1–10	3.55	6.04	3.74
	11–20	1.58		
	21–31	.91		
August	1–10	.89	4.66	4.11
	11–20	3.64		
	21–31	.13		

Some changes have been made in the project for improving Shade tobacco by breeding and selection, a project in which the Substation is cooperating with the Shade growers and with the Genetics Department of the Station. During 1940–41, the work was under the direct supervision of V. C. Brewer, Jr. Mr. Brewer entered the Army in the spring of 1942 and was superseded by C. E. Nicholson. The field plots, formerly located on the Brewer plantation in East Hartford, are now in Windsor on the plantation of the Imperial Agricultural Corporation. The research committee for the Tobacco Growers is now composed of George Gershel, Walter Edwards, Charles Griffin and G. L. Munroe.

The most important addition to the physical equipment of the Tobacco Substation in 1942 was a three-car brick garage. This is built in the same style of architecture and of the same type of brick as the main office building near which it stands. In one end is located a workshop for repairing and servicing the truck and tractor housed in the garage.

A new greenhouse was also built near the main building and will be heated by the same furnace. The head house and side walls are constructed of brick to harmonize with the other buildings. This greenhouse will be useful for continuing some projects through the winter.

The Tobacco Substation is cooperating with the U. S. Department of Agriculture and experiment stations in other states in an effort to find crops which can be grown here for rubber production. The Russian dandelion, *Taraxacum kok-saghyz*, variously treated, was grown and harvested in the fall. Analyses for percentage of rubber content have not been completed.

As a food crop that can be substituted for or rotated with tobacco on Connecticut tobacco soils, considerable attention has been given to potatoes. We have been particularly interested in determining the value of two-year rotations of potatoes with, not only tobacco, but also corn or grass. The rotations have proved superior to continuous potato culture.

Sandy tobacco soils are ideally adapted to growing sweet potatoes. For the last 10 years the Station has conducted tests on quantities and kinds of fertilizer, varieties, storage practices and methods of preventing stem rot. In view of threatened food shortage, tobacco growers might well consider the advantages of growing a few acres of sweet potatoes.

PLACEMENT OF FERTILIZER FOR TOBACCO

T. R. Swanback

The problem of fertilizer placement is receiving attention for a variety of crops. Modern row application refers to the placement of fertilizer in bands on each side of the row, a short distance away from the plants and at specified depths. Special machinery is used for this purpose, a fertilizer distributor combined with a planter.

Row application of fertilizers is practiced for most types of tobacco except cigar leaf. Since row or band placement is considered by many a more efficient means of feeding plants, individual farmers and this Station several years ago made some preliminary trials on this type of tobacco. In 1940 a two-acre field was set aside for placement tests at the Tobacco Substation in Windsor.

The field was divided into 48 plots, providing quadruplicate tests for each treatment. The purpose of the experiment was three-fold, namely: (1) to determine the effect of placing all the fertilizer in rows or bands, in comparison with broadcast; (2) to see whether a saving of fertilizer might be secured through row fertilization, and (3) to determine the effect of applying fertilizers at the time of planting, versus 10 days earlier.

The fertilizer used was a commercial 8–4–8, a grade which approaches the character of mixtures commonly used in The Valley. Twenty-five hundred pounds to the acre was the maximum applied, either in bands or broadcast. One-third of the number of plots received this amount of fertilizer, while another third received one-eighth less, or 2,188 pounds per acre, and the remainder one-fourth less, or 1,875 pounds. The row applications were made with a commercial fertilizer distributor attached to a tobacco planter. The machine was adjusted to place the material in bands a distance of 3 inches from the row on each side of the plants and at 3 inches depth.

There follows a preliminary report on the three years' results from these trials. They are published at this time as a contribution to war economy measures rather than as final conclusions. A summary of the results from these experiments is found in Table 3.

TABLE 3. SUMMARY OF THREE YEARS' RESULTS FROM PLACEMENT-OF-FERTILIZER TESTS

Fertilizer application	Year	Yield pounds per A.	Grade index	Crop index	Relative crop value[1]
Time:					
Ten days before planting ..	1940	1,752	.367	642.98	100.0
	1941	2,132	.435	927.42	
	1942	1,763	.387	682.28	(755.37)
At time of planting	1940	1,741	.375	652.88	103.5
	1941	2,171	.433	940.04	
	1942	1,790	.420	751.80	(781.57)
Method:					
Broadcast	1940	1,713	.356	609.83	100.0
	1941	2,140	.415	880.10	
	1942	1,701	.355	603.86	(700.59)
In bands on each side of tobacco row	1940	1,780	.387	688.86	119.2
	1941	2,162	.453	979.39	
	1942	1,849	.452	835.75	(835.30)
Amount:					
1,875 pounds per acre	1940	1,722	.348	599.26	87.8
	1941	2,106	.424	892.94	
	1942	1,754	.391	685.81	(726.0)
2,188 pounds per acre	1940	1,717	.367	630.14	91.0
	1941	2,140	.435	930.90	
	1942	1,747	.398	695.31	(752.12)
2,500 pounds per acre	1940	1,809	.405	732.65	100.0
	1941	2,208	.442	975.94	
	1942	1,827	.422	770.99	(826.52)

[1] Weighted three-year average based on average crop index given in parentheses.

Considering first the time element, it seems to be of some importance whether fertilizer is applied at the time of planting or 10 days earlier. A better grading was obtained in 1942 with the delayed application. In the three-year period delayed applications gave an acre return value, 3.5 per cent higher than when fertilizers were applied earlier, as indicated by the Relative Crop Value (100 vs 103.5), regardless of the method of application. Some burning of the roots on young tobacco might be expected and often has been observed when fertilizers have been applied too close to the time of planting. However, the results favor delayed application, this minor setback from burning apparently being more than compensated for by good timing.

Broadcasting fertilizer, which is the common practice for tobacco in the Connecticut Valley, admittedly is wasteful. The present trials have shown that better grading may be obtained by placing fertilizers in bands on each side of the rows. In a season with abundant rainfall, as in 1942, both yield and grading are superior with row applications as compared with the old method. In the three-year period row applications produced 19.2 per cent higher in acre return value than did broadcasting. The corresponding percentage for the 1942 season was 38.4, which further emphasizes the value of row application in a wet season.

A saving in fertilizers beyond the generally established norm for tobacco in the Connecticut Valley (200 pounds nitrogen, 100 pounds phosphoric acid and 200 pounds potash per acre) without reduction in yield and quality, is apparently not feasible, even by means of row application. In Table 3 it is found that if the amount of fertilizer applied per acre is reduced by one-fourth, the acre return value is reduced by more than 12 per cent. Reducing the amount by one-eighth decreases the value by 9 per cent. The reduction is due to both lower yields and grading.

As pointed out, these experiments have been carried on for too short a time to warrant general recommendations. At this time, however, enumeration of some of the advantages of row application may be of value to the stalk grower. The method saves time and labor as fertilization and planting are done simultaneously. The grower is more independent of weather. Unfavorable weather may delay setting of tobacco. If land is fertilized too far ahead of planting, loss of plant food must be expected in the case of excessive rains. Row application, or more precisely, placing the fertilizers in bands, results in a more even distribution of plant food. This is particularly important on "slow growing fields."

POULTRY MANURE AS A TOBACCO FERTILIZER

Poultry raising has increased greatly in the tobacco growing sections of the State in recent years. With the supply of manure more abundant, many growers have asked whether it could be used for fertilizing tobacco and what effect it would have on yield and quality of the leaf. Since there seems to be a dearth of experimental evidence to answer these questions, two field plots were fertilized in 1942 with 10 tons per acre of fresh chicken manure with the addition of 300 pounds of cottonhull ash to bring the potash content up to the standard 200 pounds. As a control, two other plots received 2,500 pounds of our regular 8–4–8 mixture, plus 700 pounds of bone meal to equalize the phosphorus.

The fresh manure, almost free of litter, was analyzed by the Station's Analytical Chemistry Department with results as follows:

	Per cent
Moisture	68.49
Total nitrogen	1.03
Total phosphoric acid	1.26
Total water soluble potash	.51
Chlorine	.17
Calcium (CaO)	1.63
Magnesium (MgO)	.28

On a dry basis, the manure would have more than double the percentage of nutrients indicated.

In the field there were no observable differences in growth of the differently treated plots.

The tobacco from the plots was harvested, cured and graded separately. At the time of sorting no differences in quality characteristics were apparent. The manure plots yielded an average of 1,808 pounds of cured tobacco to the acre; the check plots, 1,828 pounds. The grade index was the same for both.

Since some growers who had used poultry manure on tobacco reported that it affected the burn of the leaf, combustion tests were made on unfermented leaves of four grades from each plot. The average fire-holding capacity of all grades on both manure plots (160 tests) was 31.3 seconds, while an equal number of tests on the check plots gave an average of 38.7 seconds. These results indicate that there might be some impairment in burning quality from the use of poultry manure.

TOBACCO DISEASES IN 1942

P. J. Anderson

Two diseases, downy mildew and Sclerotinia-Botrytis disease, were of major importance in 1942. These are discussed in some detail later in the report. Others were either absent or of minor importance. Wildfire failed to appear at all. Blackfire or angular leaf spot was reported from a few seed beds. Pythium bed rot was found in many beds but was not very destructive. Rootrots were not reported. Mosaic was less prevalent than usual. Only a few cases of hollow stalk and sore-shin were found. Ring-spot, streak and other rare diseases were only occasionally seen.

Further Experiments on the Control of Mildew

Downy mildew, or blue mold, caused more serious losses in seed beds in 1942 than at any time since its first appearance here in 1937. The seed bed season was marked by long-continued periods of rainy cool weather which are favorable for the development of mildew. During these periods many beds were completely ruined, causing a shortage of plants for setting out the planned acreage. On the other hand, most growers who had well-constructed beds and fumigated them properly were remarkably successful in saving their plants.

During the last five years the Experiment Station has tested a long list of fungicidal chemicals and tried many methods for the control of mildew. Until 1942 the only materials found effective and practical were benzol and paradichlorobenzene (designated in the remainder of this article as PDB), used as fumigants and vaporized in tight beds. Although it has been thoroughly demonstrated that either material will control mildew if properly applied in well constructed beds, the methods of application involve certain objections which have prevented their universal adoption. Some of these are:

1. Poorly constructed beds are difficult to fumigate because the vaporized gas escapes too rapidly through the holes.

2. Poor ventilation, resulting from keeping the beds closed at night or on cloudy rainy days, may cause the plants to be tender.

3. Fumigation, which must be done at night, often means overtime work for the workers.

4. If either of the materials is left too long in the closed beds on a warm bright day, the plants may be seriously injured.

5. If benzol is spilled on the plants, it kills or damages them severely. PDB may burn the leaves if many crystals fall on them.

6. Benzol is highly inflammable and its use involves some risk. For the same reason, dealers in agricultural supplies do not like to keep it in stock.

7. Benzol and PDB are comparatively expensive if used in sufficient dosage through the season. Moreover, the war has limited the supply of both.

Obviously, an effective fungicidal spray to supplant the fumigation method would be a distinct advantage. In our search for a fungicide, new materials have been tested every year. Some of them have given partial control or, at best, have retarded the disease but none approached the effectiveness of benzol or PDB. In the winter of 1941–42, however, we began tests with what appears to be a more promising material. This is ferric-di-methyl dithiocarbamate, known commercially as Fermate.

Greenhouse Experiments with Fermate

Fermate is a sooty powder. In the experiments described here it was thoroughly stirred in water at the rate of 1½ pounds to 100 gallons of water. In early trials an equal quantity of lime was added, as recommended by the manufacturer, but later the lime was omitted and apparently made no difference in the results. No spreading or sticking agent was used. The spray was applied with a small hand sprayer which gave a fairly fine mist. Drops collected on the leaves, however, and the result was an uneven coat of Fermate on drying.

The tobacco seed was sowed in 10-inch porous pots. Treatments were begun when the plants were a quarter of an inch high. Two to four crocks of plants, each containing 200 to 400 plants, were used for each treatment. During the winter, four crops of plants were given treatments varying in rates of application and intervals between sprays. All were infected with mildew by atomizing fresh spores of Peronospora over the plants and keeping them in the moist atmosphere of an inoculation chamber for two or three days. The disease usually appeared on the unsprayed plants in about seven days. Under greenhouse conditions with a high rate of moisture and a temperature of 60° to 70° F., infection became severe on unsprayed plants. In most cases the complete batch of plants became infected and died.

Results of Fermate experiments in the greenhouse showed that:

1. The safest interval between applications is about four days. Longer periods are less effective.

2. Spraying at four-day intervals at the rate of 1½ pounds of Fermate to 100 gallons of water gives 95 to 100 per cent control. None of the sprayed plants in the experiments died from mildew.

3. Best control is obtained when the spray is applied before infection. Spraying plants already showing the disease is not so effective.

4. Spray injury is never serious enough to stunt growth to a noticeable degree. Appearing as chlorotic spots in some of the leaves, yellow "chits" and a narrowing of some of the young leaves, it occurred in some of the tests but not in others.

5. Plants continue to grow normally after repeated heavy applications of Fermate. The sprayed leaves are darker green than the unsprayed.

Fermate in Seed Beds

Fermate was compared with other treatments at the Station and also in cooperative experiments on beds of five growers of Shade tobacco. The beds at the Station were inoculated with fresh mildew spores but this was not permitted in those of the growers.

Control was good in the Station beds until the spray schedule was interrupted late in the season by a 10-day period of rain. During this time most of the Fermate was washed off the leaves and no further applications were made. Near the close of this period mildew got well started and necessitated fumigating with PDB.

The five Shade growers all reported success with Fermate. However, from an experimental viewpoint, the tests were inconclusive because three of the growers fumigated their other beds so carefully mildew was practically non-existent anywhere. The fourth grower had considerable mildew in his other beds but only a very small amount in the Fermate beds.

The beds of the fifth grower, consisting of about two acres, furnished an excellent demonstration of the value of Fermate because no treatment was used on any except four or five that had been sprayed with Fermate. Mildew was visible on 75 to 100 per cent of the plants in the unsprayed beds at the close of the plant bed period in mid-June. Only a trace of mildew could be found on the sprayed beds. Because their dark green color contrasted with the yellow leaves of the diseased plants, the sprayed beds could be picked out at a distance by even an inexperienced observer.

The results of the 1942 tests give hope that we may at last have an effective spray against mildew. Before final conclusions are drawn, however, tests should be repeated over a period of several years. This would give an opportunity to see how Fermate performs under a variety of conditions.

Flordo

This copper-soap spray was described in last year's report (Bul. **457**, p. 250) which stated that, though Flordo did not give entire control, it proved better than any of the other liquid sprays tried. Experiments were continued in the greenhouse in the winter of 1942 and more extensively, later, in seed beds. In all tests, it delayed the appearance of mildew for several days or a week but, with weather conditions favorable for the spread of mildew, all the plants were finally infected. However, fewer of them died than in the unsprayed blocks. Although this material has some fungicidal value, its effectiveness in the control of mildew is so far inferior to that of Fermate, it would hardly seem worthwhile for growers to continue its use.

Copper Dusts

Growers have told us of success in controlling mildew by dusting plants with commercial copper mixtures. Although previous tests have indicated such mixtures to be of little value, we included two, Basi-cop and Copper-Hydro, in 1942 greenhouse tests. Plants were thoroughly dusted with the materials every four days and, after two treatments, were inoculated with disease spores. The appearance of mildew was somewhat delayed, but when it started it proved almost as damaging as on the check plots. Almost all of the plants finally died from mildew attack in spite of repeated treatments. No results to date warrant the recommendation of copper dusts.

Better Methods of Using PDB

PDB as a fumigant in beds has been generally effective against mildew and its use has been adopted by most growers. There are, however, certain conditions which result in failure. This is particularly true when night temperatures are too low to produce sufficient vaporization of PDB.

Experiments of the last three years have shown that this failure may be overcome by providing for a more thorough distribution of the PDB crystals than is obtained by putting them in the small wire baskets commonly used.

Two methods of distributing PDB on cheesecloth, supported under the glass sash but above the plants, have proven highly successful even at low temperatures. By the first method (Figure 1) an 18-inch strip of cloth running the length of the bed is supported over the center. In the second (Figure 2), the crystals are scattered on light cheesecloth frames which cover about one-third of the bed area. The frames are supported on spike nails driven into the sides of the bed and may be removed during the day. It is not so convenient to remove the long strip of cloth, mentioned above, but this is not necessary if the sash is raised on bright days. Both methods have given complete control under conditions where the basket method gave only partial control.

Sclerotinia and Botrytis Diseases in Connecticut

After a week of rainy weather in mid-August a leaf rot and stalk canker became prevalent and caused considerable damage in the Shade tobacco fields. To answer the many inquiries by concerned growers, the writer undertook an investigation. Although occasionally seen here, the disease had not recently been common and, so far as is known, no study of it had been made in America.

Preliminary investigation indicated that the trouble was caused by a parasitic fungus of the genus Sclerotinia. Further search showed the presence in many of the diseased leaves of another parasitic fungus of the genus Botrytis. It was concluded that two different diseases were present, their symptoms being almost indistinguishable, but caused by two distinct and unrelated fungi. This strange parallelism, noted previously for these two pathogens on other plants, had never been recorded by American pathologists on tobacco. Some investigations had been made abroad, however, where the diseases seem to have attracted more attention.

The writer has found one or both of these fungi before and has recorded their occurrence in the annual reports. However, the present investigation has crystalized past observations and given proof of the importance, not previously recognized, that these two fungi bear in tobacco pathology.

Descriptions follow of the varied manifestations of the disease of tobacco caused by these fungi, of their relationships and of their courses of development throughout the season.

From a symptomatological standpoint, these diseases manifest themselves in the following phases: (1) leafspot and rot in the field, (2) stalk canker in the field, (3) stalk rot in the shed, (4) pole rot and (5) bedrot or damping off. All are closely related but for convenience will be discussed separately in the order listed.

Leaf Spot or Leaf Rot

Symptoms. On the leaf, the disease almost always starts where the corolla of a blossom has fallen and remains adhering to it. The blossoms are removed from the open-field binder tobaccos but are allowed to develop in great profusion in the Shade type; this accounts for the prevalence of the disease in Shade tobacco and its rare occurrence in open-field types. The disease is never found on the first priming of leaves because they are removed before the blossoms begin to fall. From the second priming up, it occurs in progressive abundance, varying, however, with the weather that prevailed the week previous to priming. The disease is dependent for its development on prolonged periods of damp weather.

As a fallen blossom withers on the leaf, the tissue directly under it takes on a dark water-soaked appearance. Then the diseased area enlarges into a circular, olive-brown dead spot. When the return of dry weather stops the spread of the spot, a definite margin is formed. The dead tissue assumes various shades of brown or may be bleached out in time to ashen gray or almost white (Figure 3). The writer has previously described this arrested dead spot as "dead-blossom leaf spot" (Bul. **386**, pp. 595–598).

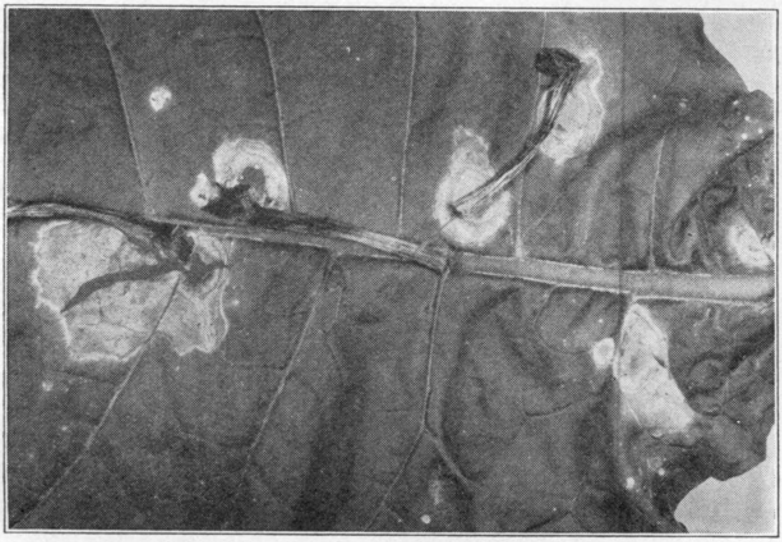

Figure 3. Typical dead-blossom leaf spot showing blossoms still adhering to the leaves.

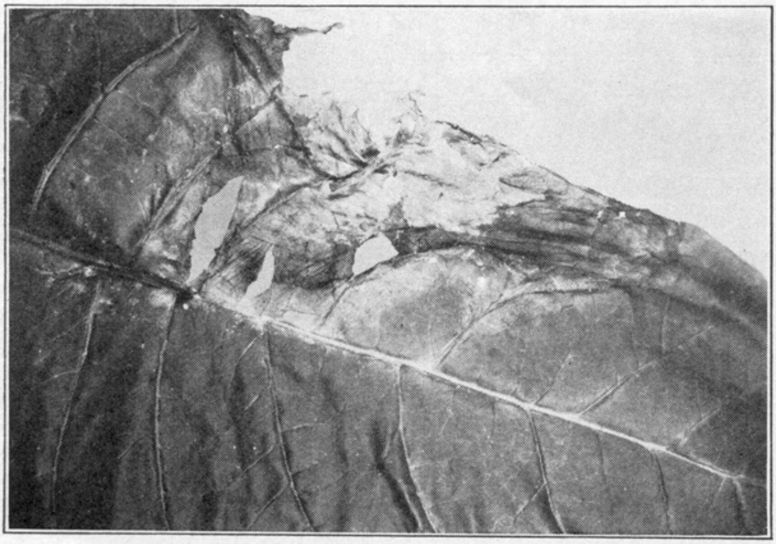

Figure 4. Sclerotinia leaf rot as it spreads in wet weather.

In a clear-cut Sclerotinia type of leaf spot the spread of the dead area is very rapid. The tissue becomes olive brown and turns lighter only with prolonged exposure to sunlight. Each return of wet weather starts a rapid spread from the margin of the old spot until large irregular areas of the leaf, or even the entire leaf, are rotted (Figure 4). The rotted leaf is very tender, and tears or falls apart when handled.

Symptoms which distinguish the Sclerotinia or Botrytis type of spot from the others are: (1) the extreme rapidity of spread and (2) the olive green color of the tender tissue. During wet weather there is another diagnostic symptom which definitely distinguishes Sclerotinia leaf rot, viz., the occurrence of cottony white tufts of mold on the affected parts, especially along the veins or in folds of the leaf. Some of the largest of these small tufts develop later into the hard black sclerotia subsequently described. No other diseases of tobacco in Connecticut develop anything resembling the white mold tufts, or sclerotia, on the field leaves. Their presence may be considered as a sure proof of the Sclerotinia disease.

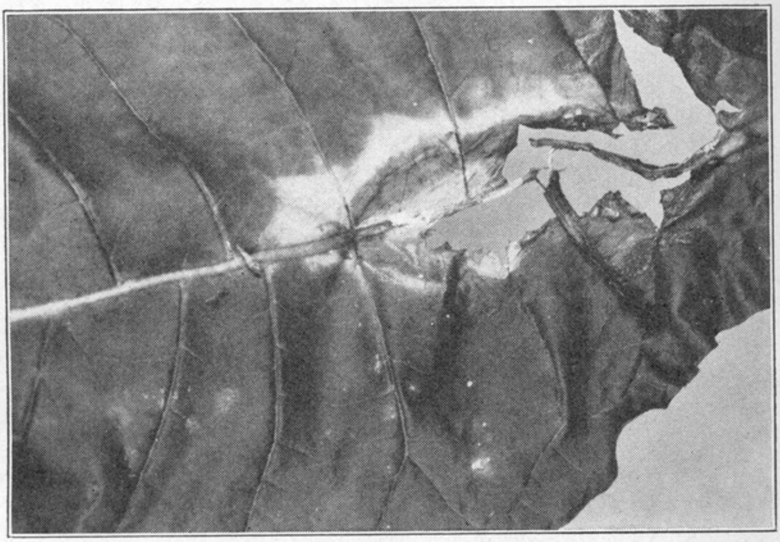

Figure 5. Vein rot in the field.

Vein rot is another manifestation of Sclerotinia disease. Sometimes a dead blossom lodges directly on the midrib, or a leaf spot may enlarge until it involves a midrib. The rot works rapidly in the midrib, softening and weakening the tissue until it can no longer support the weight of the leaf (Figure 5). Sooner or later the leaf droops and dies. Frequently the rot works down the midrib to the stalk. The yellow drooping leaves are quite noticeable in the field and are another sure sign of Sclerotinia disease. The white mold tufts and black sclerotia occur commonly along the diseased midribs in damp weather but may be absent altogether in dry periods.

Fungi associated with leaf spot and rot. During the investigations described in our report for 1935 (Bul. **386,** pp. 595-598) a number of species of fungi were found on and isolated from the dead blossoms and from the spots under them. *Alternaria tenuis* was most common, next *Botrytis cinerea,* then *Cladosporium herbarum* and, least frequent of the four, *Sclerotinia sclerotiorum.* Others were only occasionally associated with the trouble and could hardly be suspected of being causal agents. Inoculation of green leaves with the most commonly found species, Alternaria, failed to produce typical spots and left considerable doubt as to the causal role of this species. It is admitted, however, that under different environmental conditions it might cause a spot. Lack of time prevented further testing of species that year.

Opportunity for more extensive isolations was offered by the severe outbreak of 1942. Dozens of isolations were made from spots in all stages of development on leaves and blossoms. Most of these gave pure cultures of Sclerotinia or Botrytis. Alternaria was found only on the dead blossoms or the oldest parts of the spots. In the youngest and smallest lesions Botrytis was most often found, while in the edges of the extensive rotted areas Sclerotinia predominated. Cladosporium was found only in a few of the older blossoms. Spores and sporophores of Botrytis developed on the old blossoms and on the immediately surrounding rotted leaf tissue. The presence of Sclerotinia was indicated by prominent cottony tufts of aerial mycelium and by dull-black sclerotia, particularly along the midribs and in folded or shaded parts of the leaves.

Because of their constant association with the trouble this year, and because neither Alternaria nor Cladosporium had shown any decided parasitic qualifications in previous tests, Sclerotinia and Botrytis were subjected to further investigation.[1]

Inoculation studies. Both fungi were readily isolated and grown in pure culture on a number of media. Potato dextrose agar was found to be the most suitable and convenient medium for further observation and work. The cultural, physiological and morphological characteristics of the two fungi were studied in detail. Since descriptions have been previously published they will not be repeated here.

Both fungi grow with extreme rapidity on the agar medium, covering the surface of the slant in about four days at laboratory temperature. The Sclerotinia cultures can be readily distinguished by the formation of the black sclerotia scattered over the surface of the slant (Figure 6). While the sclerotia are still young, they bead the surface with clear liquid. Botrytis can be distinguished from Sclerotinia by its more abundant aerial mycelium among which the tiny clusters of spores, first white and then smoky gray, may be seen with the naked eye. It produces sclerotia also but they are in an almost continuous band where the agar comes in contact with the glass. Thinner and crust-like, they are convolute on the surface and do not have

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[1] The pathogenicity of other species of fungi has not been sufficiently investigated to warrant publication of results at this time.

either white centers or exudations of clear liquid in drops such as one sees in Sclerotinia sclerotia.

To find out whether either or both of these fungi are pathogenic on leaves, small blocks of agar containing mycelium of the fungi were taken from the culture tubes and placed on the surface of green tobacco leaves. In one set of trials the leaves were removed from the plant, washed thoroughly in water and placed in large moist chambers before the addition of agar blocks. Within two days dark watery spots appeared on the leaves under the agar. At the end of four days all the spots were an inch or more in diameter and the tissue was completely rotted. The rot continued to spread until the entire leaf was involved and the tissues so completely decomposed that the leaf could not be handled without dropping apart. The development of the rot spots was the same, regardless of which of the two fungi was used for inoculation, until the tufts of mycelium, forerunners of sclerotia, appeared on the leaves inoculated with Sclerotinia. After about a week, isolations were made from the margins of the spots and, invariably, pure cultures of the fungus which had been used in the inoculum were recovered.

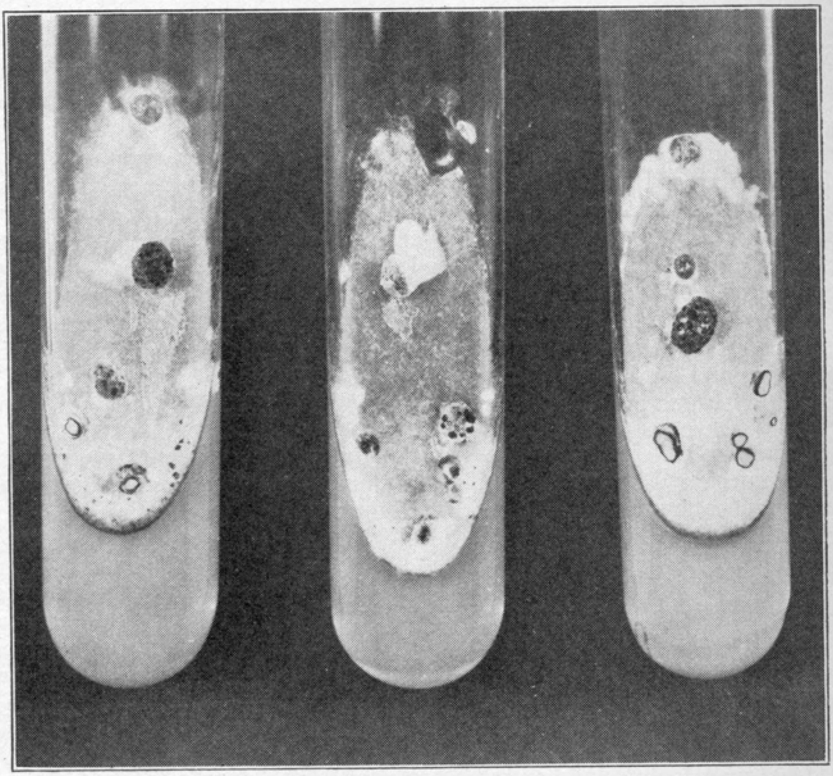

Figure 6. Pure cultures of Sclerotinia showing the black sclerotia and white cottony mycelium.

In a second set of inoculation trials the agar blocks were placed on the leaves while still on the growing plant. The entire plants, about 2 feet tall, were covered with a large glass inoculation chamber to duplicate moist conditions such as occur in the field during an outbreak of this trouble. The results were the same on the leaves of growing plants as they were on those which had been removed. Rots such as one sees in the field were produced by both fungi. After a few days the plants were removed from the inoculation chamber. Under drier conditions the rate of spread of the spots diminished or stopped entirely.

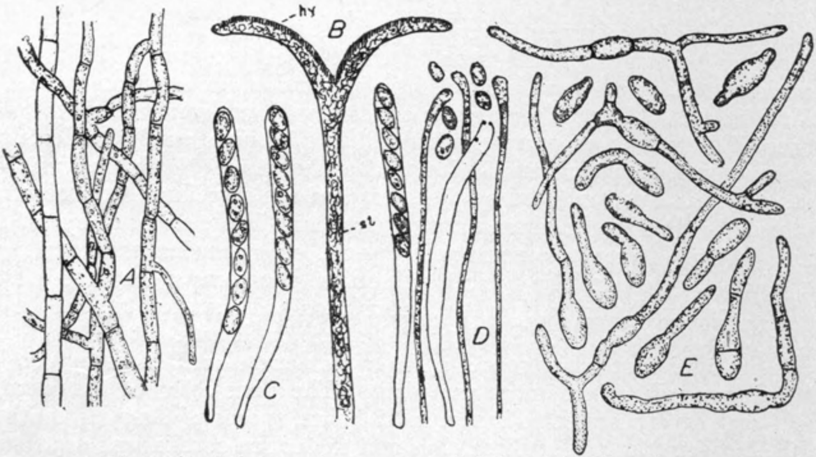

Figure 7. Microscopic structure of *Sclerotinia sclerotiorum.*

A. Threads of mycelium from an agar culture, magnified 100 diameters.
B. Diagrammatic longisection of an apothecium, magnified 20 diameters, hy = the hymenium where the asci and paraphyses are located, st = stipe or stalk.
C. Asci with ascospores, x 75.
D. Paraphyses and an empty ascus.
E. Stages in the germination of the ascospores, x 100.

These experiments, repeated several times with some modifications, showed conclusively that either of these fungi under the conditions described is pathogenic and capable of causing a rapid rot of normal green leaves.

Role of the blossom in infection. Next, the leaves were inoculated with spores of Botrytis. Spores from pure cultures were suspended in water and sprayed on the leaves which were then kept under moist conditions for a week. All results were negative. Later, leaves were inoculated with viable ascospores of Sclerotinia, but again no infection took place. The spores germinated freely (Figure 7E) but the germ tubes died without penetrating the living cells of the leaf. This striking difference in infection power between the spores and mycelium in nutrient agar suggests that the strength of the fungus must first be built up by a period of saprophytic growth before it is able to infect. Or, possibly there might be a "mass action" force in a quantity of mycelium that would be lacking in germinating spores.

Such a theory explains the role of the fallen blossoms in starting the rot spots on the leaves. When the blossoms begin to wither and drop to the leaves below, they represent dead tissue and, as such, are a suitable medium for growth of the fungi. Thus they have the same function as the blocks of nutrient agar used in the inoculation experiments discussed above.

A number of experiments were made to test the ability of Botrytis and Sclerotinia to thrive on the corollas of tobacco blossoms. In the first experiment, blossoms were sterilized by immersion for a minute or two in boiling water. Then, under aseptic conditions, some were inoculated with spores of Botrytis and others with small bits of mycelium of Sclerotinia, the ascospores being unavailable for tests at that time. Both fungi thrived on this medium, as indicated by the spread of the mycelium. Spores were produced by Botrytis in three or four days and, later, sclerotia by Sclerotinia. When infected blossoms were placed on green leaves under moist conditions they caused the typical rot spots. Blossoms picked fresh from the field and not sterilized before inoculation with spores, turned brown within 24 hours and produced typical spots on leaves. In a final test, drops of water containing spores of Botrytis were placed on living corollas without removing them from the plant. The pink color of the blossom under each drop changed to brown in 18 hours, spores of Botrytis appeared on the blossoms in two or three days and the entire corolla was discolored. When these blossoms were placed on green leaves they caused typical rot.

Later, when fresh ascospores of Sclerotinia were available, these were used to inoculate corollas both on and removed from the plant. The corollas turned brown where inoculated and the tissue was full of mycelium. Two days after inoculation the flowers were transferred to fresh green leaves. In two days rot spots had spread around the blossoms.

These experiments make clear the role of the blossoms in starting leaf rots. Although the spores of both species are incapable of directly infecting the leaves, they readily infect the corollas of the blossoms and produce a mass of mycelium in them. When the dead corollas lodge on green leaves, the mycelium is able to pass from the dead flower into the living tissue. Living as a parasite, it kills the cells by the secretion of a toxin and causes the leaves to rot.

The necessity of this saprophytic "build-up" before infection may be demonstrated further. Although practically all of the leaf spots start around adhering corollas, a few start in a different manner. In harvesting the lower leaves, some of the upper leaves may be accidentally broken. Growers call these "broken backs." Occasionally a disease spot may be found starting from the broken midribs of such leaves. Here, again, the injured tissue offers an opportunity for a saprophytic start. Finally, some spots may be found where a rotted leaf lies directly on or against a healthy one, the new spot appearing at the point of contact. In the picking baskets where the leaves are left "padded" together we have seen the rot pass from one leaf through a whole pad in a few hours. There can be no doubt that spores of both of these fungi are deposited in great numbers on the leaves of tobacco[1] at picking time. Allowed abundant opportunity for germination and infection of the healthy leaves, infection occurs only where there are blossoms on the leaf or where there is dead tissue for saprophytic nutrition.

Stalk Canker in the Field

Symptoms. The outstanding feature of the 1942 outbreak was the abundance of stalk cankers in the fields of Shade tobacco during late August. Almost without exception these cankers start where the base of a leaf is attached to the stalk. The rot spreads down the leaf, especially along the midrib. When it reaches the stalk, it begins to spread both up and down. In its youngest stages, the little canker is watery and olive-brown like the young leaf lesions. But it soon turns to a cinnamon brown, the color of a cured tobacco leaf. With age it bleaches out to a straw color or almost white at the center, remaining dark brown at the margin. The canker is oval in outline, spreading more rapidly in a vertical direction than around the stalk (Figure 8). Slightly sunken, it frequently shows con-

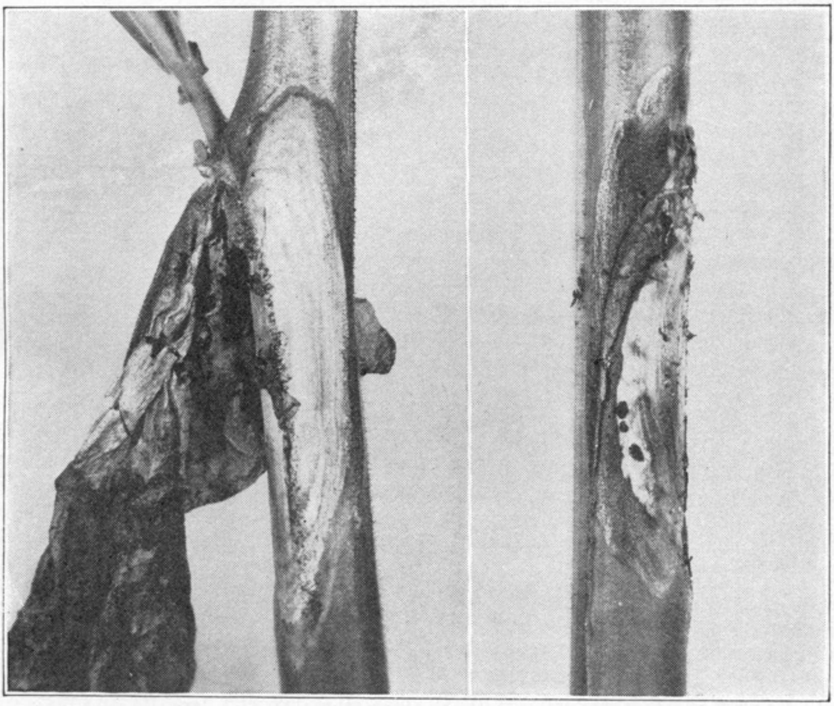

Figure 8. Two stalk cankers in field. Stalk on left shows the drooping leaf, through which fungus entered the stalk, and the sucker in the axil, not yet affected. Three black sclerotia appear on the surface of the canker to the right.

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[1] R. E. Smith (Phytopath. **21**:407–423, 1931) in an investigation of a rot of apricots caused by *Sclerotinia sclerotiorum* demonstrated that at the proper season of the year the spores of this fungus occur in great numbers on the leaves of all kinds of plants without causing infection.

centric rings and a definite margin. In wet weather white tufts of mycelium, which later develop into sclerotia, appear at points on the surface. The sclerotia, dull black outside and white inside, are oval and cushion-like. Flattened against the stalk, they grow to a quarter of an inch long. They seem to originate mostly just under the epidermis which they push up and rupture.

The canker spreads around the stalk until it girdles it. Then the leaves above the canker turn yellow, droop and die. If the stalk is split open it will be found either hollow or with brown pith. In the cavity are black sclerotia, more elongated and irregular in shape than those on the outside. They are loosely attached so that, in an old canker, they rattle around when the stalk is shaken. Irregular patches of white mycelium line the walls of the cavity. The woody cylinder of the stalk does not disintegrate but remains hard and intact.

Isolation of the causal fungus. Isolations were made from the surface of the canker, from the woody cylinder, from the pith and from the interior of the sclerotia. All gave pure cultures of just one fungus, Sclerotinia. Botrytis was not isolated from any of the cankers investigated though it is possible that more extensive search might show that these cankers also occur. The cultural and morphological characteristics of the fungus isolated from the cankers were the same as those previously determined for the leaf pathogen.

Inoculations. In the first tests, short pieces of fresh tobacco stalks were sterilized on the surface by (1) dipping in boiling water, (2) washing in alcohol or (3) washing in mercuric chloride solution and then sterile water. These pieces were then introduced into tubes of pure cultures of Sclerotinia. In four or five days the pith and surface tissues were rotted to a watery mush. The results were the same, but did not occur quite so rapidly when Botrytis cultures were substituted.

In a second set of tests, green leaves of growing plants, 2 feet tall, were inoculated with Sclerotinia under a glass inoculation chamber. Rot spots developed on all of these leaves within a week. After the plants were removed from the chamber, the rot continued to spread down the midribs until it reached the stalks. Here it started cankers and the plants began to wilt. In 15 days all the plants had died. Reisolations from the stalks gave pure cultures of Sclerotinia.

Plants inoculated in this way with Botrytis developed the same kind of leaf spots but these failed to spread to the stalk and none of the plants died.

These experiments show that Sclerotinia can run from the leaf into the stalk, kill and disintegrate its outer tissues, pass through the woody part without rotting it and then rot the pith. All the stalk above the canker then dies.

Stalk Rot in the Curing Shed

After stalk-cut tobacco is hung on the poles in the shed to cure in August or early September, the stalks normally remain green and little changed in appearance until the fully cured brown leaves are stripped off in October or November. But there are always some stalks, and occasionally many, which do not remain green because they are attacked by a rot before the leaves are cured. This may result in serious damage because the leaves do not cure properly on a rotted stalk and the rot often passes out into the leaves causing their complete ruin.

During the curing season of 1942, when this trouble was prevalent, a careful study was made of the stages of its development and its causal organisms. In every instance one of two fungi, Botrytis or Sclerotinia, was found to be present in the affected tissues of the stalk and on the surface.

The first stages of the disease were observed in the shed in early September. Regardless of whether Botrytis or Sclerotinia is the cause, slimy rot starts at or near the tip of the stalk and works down toward the butt, involving from a few inches to the greater part of the stalk. "Slippery stalk" is a common name applied to this stage because, when the stalk is grasped, the rotted outer layer slips off the underlying woody cylinder. This soft tissue no longer holds together. The pith is also affected in the manner previously described for stalk canker in the field.

The rot runs from the stalk into the mid-veins of the leaves as long as they are still "sappy" enough to support the fungi. The veins become completely disintegrated except for the vascular bundles which fray out like the strands in the end of a rope. In this condition the web of the leaves separates from the midveins. Many of the leaves drop off the stalk and fall to the floor of the shed. If the stalk is killed early in the curing season the leaves stay green and "hayed-down" and never have a proper cure.

The grower often fails to notice the rot until he takes down the tobacco and strips off the leaves. Then in the final stage, Botrytis rot is quite different from that of Sclerotinia.

In Sclerotinia rot, there are irregular patches of cottony mycelium, either scattered or covering large sections of the brown stalk. Dull-black irregular-shaped sclerotia from the size of a buck shot to that of a pea are scattered or clustered on the surface. The epidermis sloughs off in patches, exposing frayed, hair-like bast strands on the outside of the woody cylinder of the stalk. The stalk is usually studded with prominent wart-like protuberances, lighter in color than the sclerotia. Appearing to originate below the epidermis, they push up and rupture it. Closer examination shows the warts to be clusters of large white crystals, the chemical nature of which has not been fully determined. Shaking the dry stalk causes the loose pellets to rattle, accounting for its common name of "rattle box." When the stalk is split open, dull black irregularly shaped sclerotia are disclosed. A small handful of them may often be emptied from one stalk. Lining the inside of the walls are large patches of white mycelium.

If the rot is caused by Botrytis, however, there are no sclerotia inside or outside the stalk, no mats of white mycelium and no crystalline protuberances. In this case the infected part of the stalk becomes covered with a thick mouse-colored mat which feels and looks like velvet. Clouds of dust-like spores come from it when the stalk is shaken or blown upon.

These two easily distinguished rots are equally damaging. The Sclerotinia type was much more prevalent in the sheds in 1942 than was the Botrytis type, but this condition might well be reversed during other seasons. The writer has not seen both of them on the same stalk; of many isolations made from the rotted stalks pure cultures of one or the other almost always resulted. A slower and less serious rot of the stalk is sometimes caused by Alternaria or Fusarium but the symptoms are quite different from those described.

Stalk rot in stalk-cut tobacco does not start from infected flowers, as does pole rot in Shade tobacco. The blossoms are removed before they are fully developed and therefore they never come into the curing shed. Observations of the early stages of the disease lead us to believe that it starts in dead wound tissue from which the tops or suckers have been removed. No inoculation experiments have been undertaken to prove this assumption, however.

The method of controlling stalk rot obviously is to keep the atmosphere in the sheds as dry as is consistent with good curing. The fungi thrive only in a moist atmosphere. Good ventilation combined with early and heavy firing should greatly reduce, if not eliminate, stalk rot.

Studies on Pole Rot. III

Pole rot is a term used to cover all types of decay of tobacco leaves while they are curing in the shed. In two previous reports[1] the writer has discussed the general aspects of the trouble and, for convenience in treatment, has designated three types of pole rot as "freckle rot," "vein rot" and "web rot."

"Freckle rot," fully discussed in the first article cited, was shown to be caused by the fungus *Alternaria tenuis*. In the investigations on vein rot, described in the second, a number of species of fungi were found in the rotted veins, including Alternaria, Botrytis, Fusarium, Sclerotinia and Cladosporium. The most common rot that year (1936) was associated with Alternaria so this type was studied and described. Since both Botrytis and Sclerotinia were prevalent in 1942, the opportunity was presented to make further observations and studies on vein rot and on web rot, associated with these two fungi.

Vein Rot. It was stated above that the rot which involves the stalks in the shed frequently runs out from the stalks into the mid-veins of the attached leaves. This appears to be the start of almost all of the vein rot cases caused by the two fungi that we have observed in stalk-cut tobacco. In Shade tobacco, however, vein rot is not so common except where it is associated with web rot. Here it always starts from an infected dead blossom or, at least, a dead-blossom spot which was on the leaf before it was taken from the field. It is not unlikely, however, that even in stalk-

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[1] Conn. Agr. Expt. Sta. Bul. **386**:600–607 and Bul. **391**:112–117.

cut tobacco infection may originate on the veins at some distance from the stalks. Since the grower usually first becomes aware of vein rot after the leaves have been stripped from the stalk, this point is not certain.

Unlike Alternaria rot, which begins after the veins have lost their green color, Botrytis or Sclerotinia rot may attack the veins while they are still green. The veins remain susceptible to rot so long as they are still "sappy." All the tissues of the veins become quickly and completely disintegrated except the fibro-vascular bundles which then resemble the ravelled threads of a cord. Affected midribs are prominent because they turn white or a light straw color. The web of the leaf drops away from the vein and the leaf falls apart on handling. Such a leaf is worthless commercially.

Some sclerotia of Sclerotinia may be found on the surface of rotted veins but not so commonly as on the stalks; frequently they cannot be found at all. Botrytis spores may occur occasionally on the veins, but more often not. Nothing except the presence of sclerotia or spores distinguishes the Botrytis from the Sclerotinia vein rot.

Isolations and inoculations. Many isolations from affected veins taken in different localities and different years have shown that Botrytis or Sclerotinia mycelium is commonly present in this early type of rot. The other fungi are found less often here but become more common in later attacks. One of them, Alternaria, has been definitely proved to cause a vein rot and there are indications that others may be responsible at times. At present, however, we are concerned only with the type caused by Botrytis and Sclerotinia.

The pathogenicity of these fungi on the midribs has been demonstrated by inoculations. In one type of inoculation, sections of green ribs were sterilized on the surface by methods described in Bul. **391**, p. 115, then introduced into pure cultures of the fungi. These fungi attack with such speed and vigor that a midrib a quarter of an inch in diameter and 2 inches long may be completely disintegrated in four days. It is reduced to a soft watery mush that collapses when touched.

In other inoculations, mycelium from pure cultures was placed on the midribs of detached green leaves kept in moist chambers. Rot spots began to appear on the veins within two or three days and enlarged rapidly both along the veins and out into the web of the leaf. Both Botrytis and Sclerotinia completely disintegrated the veins under these conditions.

In a third type of inoculations corollas of blossoms, first sterilized and then inoculated with Sclerotinia, were inserted between the green leaves of Shade tobacco suspended on a lath in an inoculation chamber. The rot spread from leaf to leaf involving both web and veins. At the end of 10 days all the leaves on the lath were affected and some of the veins were so rotten that the leaves dropped from the string to the floor. Sclerotia had developed on the surface of the midribs.

It is apparent from all these tests that both Botrytis and Sclerotinia are virulent pathogens on the veins of leaves in the early stages of curing and under favorable conditions may cause extensive damage in both stalk-cut and Shade tobacco. The extent of the damage caused by the activity of these two fungi in the mid-veins has undoubtedly been much underrated.

Green Web Rot. The name "web rot" designates an effused type of rot affecting the tissues or web of the leaf that is between the veins. (The commonly used term "web" is probably derived from its resemblance to the web between the toes of a water fowl.) Web rot is different from freckle rot in that it does not appear as clustered numerous small spots but spreads in a broad continuous sheet over a considerable part, or even all, of the leaf surface. Unlike vein rot, it affects the web, although the same organisms are sometimes responsible for both. Web rot is the most destructive of the three types of pole rot.

Study of pole rot year after year in different crops and under varying conditions shows there are several kinds of web rot which differ in appearance, behavior and effect. This fact probably accounts for the confused state of our knowledge, as reflected in literature on the disease. In the present account the writer will discuss only that type of web rot which he has designated as "green web rot."

There are two reasons for calling this web rot "green." First, it is the only type that starts on the leaves while they still have their normal green. Other more common types begin after the leaves have turned yellow or are passing into the brown stage. Second, the affected part, even when completely dried, is of some shade of green, usually an olive green or greenish brown. This greenness is retained because of the rapidity with which the leaf tissues are killed; apparently there is not sufficient time for the green chlorophyll to break down.

Green web rot affects both stalk-cut and Shade tobacco. In 1942 it was particularly destructive also to Primed Havana Seed, a type produced by a few growers this year. The role of the faded blossoms was quite striking on these plantations; hence the common name "blow-rot," used by growers.

In Shade leaves it usually starts from a dead blossom or an affected leaf brought in from the field, and spreads quickly by contact through adjacent leaves. In its early stages it is a watery slimy rot, no different from the field leaf rot which has been described. In fact, the latter might well be called "pole rot in the field." After the leaves have dried in curing, the affected parts are either a vivid green ("hayed-down") color contrasting sharply with the normal leaf, or darker in color, frequently even a greenish black, and with a distinct but usually irregular margin. Having no distinctive shape they may cover any portion or all of the leaf. The affected part is dull, lacking the luster or finish of a normal cured leaf. The tissue is brittle, breaking into fine fragments with the least handling. It does not become soft like the other parts of the leaves when placed in a damp atmosphere; if moistened, it drops apart easily since it lacks cohesion. Such leaves have no value.

Causal fungi. In mild cases, no fungi are found on the surface, although examination under the microscope shows the tissues permeated with a stout hyaline septate branched mycelium (Figure 7A). In more virulent cases, especially where leaves are matted together, white patches of mycelium are found on the surface. Later the cottony bunches produce black sclerotia, showing the presence of Sclerotinia. Sometimes tufts of spores and sporophores of Botrytis are observed in the later stages of rot. Isolations from the rotted tissues invariably give cultures of either Botrytis or Sclerotinia. When blocks of agar from pure culture tubes of these fungi are placed on single leaves in a moist chamber or inserted between closely hanging leaves on a lath, the same type of rot is reproduced. Thus the capacity of either of these fungi to produce green web rot in the curing shed has been fully established.

It is interesting that 50 years ago Behrens[1] in Europe described this same type of pole rot and showed its association, usually with Sclerotinia but sometimes with Botrytis. (His *Sclerotinia Fuckeliana* and *Scl. Libertiana* are the same two species as we have discussed here under the names of *Botrytis cinerea* and *Sclerotinia sclerotiorum*). Just previous to Behrens' publication Sturgis[2] in Connecticut had made a study of the disease and decided it was caused by *Botrytis longibrachiata* Oud., a species which he considered quite distinct from *B. cinerea*. But he later decided that it was caused by bacteria. During the last 50 years there are occasional references to Botrytis and Sclerotinia rot in European literature but no reference to its occurrence in America. It is not known whether this was due to the absence or only rare occurrence of the disease in America, or to lack of sufficient investigation by American pathologists. The writer is inclined to believe that the latter is the true explanation.

Bed Rot or Damping-Off

Bed rot is a term used by tobacco growers to designate rotting off of the stalks of seedlings still in the seed bed. This occurs when the seedlings begin to crowd and are approaching the size for setting in the field. Damping-off is a less-used term.

This trouble in seed beds in Connecticut was first mentioned by Clinton in the Station Report for 1905. The following year he gave a more complete description of it, changing the name to "stem rot" and ascribing the damage to a species of Sclerotinia which he said resembled *Sclerotinia Libertiana*, the cause of lettuce drop. The photograph of the fungus in culture which he published in the report for 1905 shows sclerotia very much smaller than those which the present writer has observed in cultures on the same kind of agar. This suggests that he may have isolated the more recently described *Sclerotinia minor* Jag. of *Scl. intermedia* Pam. Clinton states that this was the most common type of bed rot at that time in Connecticut.

Bed rots of tobacco are not uncommon. The writer has examined hundreds of cases during the last 15 years, but only occasionally has he found any caused by Sclerotinia. Species of Pythium are the most common

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[1] Behrens, J. Trockene und nasse Faule des Tabaks. "Der Dachbrand," Zeit. f. Pflanzenkrankheiten **3**:82–90. 1893.

[2] Conn. Agr. Expt. Sta. Rpt. for 1893, p. 84.

pathogens of bed rots here. Others are caused by Rhizoctonia or by bacteria. The almost universal practice of sterilizing the soil before sowing the seed probably accounts for the rare occurrence of Sclerotinia in recent years. Sterilization of the soil was not generally practiced 35 or 40 years ago.

Symptoms. The symptoms of bed rot caused by Sclerotinia are quite similar to those caused by other pathogens, but there are a few differences that may be noted by a careful observer.

The trouble first appears when the plants are 2 inches or more high and in the parts of the bed where growth is thickest. The plants die off in rounded spots from a few inches to a foot or more in diameter. The stalks of the seedlings are first attacked by a slimy rot. The rotted part does not turn dark brown, as in other types of bed rot, but remains a light straw color. As the rot travels up the stalk it attacks each leaf in succession and runs out into the blade. As it falls or droops, the leaf comes in contact with healthy leaves of adjacent plants. These in turn become infected and form a perfect bridge for the fungus to pass from the stalk of one plant to the stalk of the next.

All parts of the plants completely disintegrate and finally collapse. When they dry out, the mass of dead and blanched leaves forms a continuous parchment-like crust over the ground. Under moist conditions the dead stalks and leaves give rise to cottony growths of aerial mycelium, sometimes a quarter of an inch high. Some mycelial threads may be seen growing over the surface of the soil but never so luxuriantly as on the rotted plant parts. Sclerotia may form on the rotted plants on the ground but they are usually not very numerous.

The spread of the rot from plant to plant in a given spot seems to be through the bridging leaves rather than the mycelium on the soil. The aerial mycelium and the sclerotia are the best diagnostic symptoms for this type of bed rot. With the return of dry atmospheric conditions the centrifugal spread of the spot stops. There is little danger of carrying the disease into the field because most plants that are affected at all fail to survive long enough to be set out.

Pathogenicity studies. In order to determine whether *Sclerotinia sclerotiorum* is capable of producing this type of bed rot and to study the stages in the development of the disease, inoculations were made. In a bed where the plants were 2 or 3 inches high and growing thickly, blocks of agar from a pure culture and containing mycelium of Sclerotinia were placed on the surface of the ground between the plants and close to the stalks. The inoculated areas were covered with large bell jars. Bell jars were also set over check areas.

Within four days after inoculation, the plants nearest the inoculum began to die. The rot spread centrifugally until at the end of two weeks all the plants under the bell jars were dead. When the bell jars were removed after the rot had already killed the central area, there was little further spread of the disease as long as the weather was favorably dry. Isolations made from the dead plants farthest removed from the point of inoculation gave pure cultures of Sclerotinia. No rot appeared under the bell jars where no agar blocks had been introduced.

A similar set of inoculations was made by using pure cultures of Botrytis. Some of the leaves that came in contact with the agar died. The rot spread from leaf to leaf in the lower leaves but it did not pass into the stalks, so none of the plants died. These negative results indicate that Botrytis does not cause a bed rot, at least not under these conditions. Moreover, of all the cases of bed rot that we have had occasion to examine during many years, we have never found any caused by Botrytis.

In North Carolina, however, Wolf[1] found that *Botrytis cinerea,* often not only kills the lower leaves but may invade the stalks of seedlings in the beds and rot them off. A similar Botrytis disease of tobacco seedlings also occurs in Java.[2] Possibly more extensive investigation may show that this species is capable of causing bed rot under our conditions also.

Summarizing our observations and investigations, we may conclude that *Schlerotinia sclerotiorum* is capable of producing a virulent bed rot under moist atmospheric conditions. This is not the common type of bed rot in Connecticut but is sometimes found, and may become severe enough to cause great damage.

Overwintering of Sclerotinia

The most characteristic feature of the fungus *Sclerotinia sclerotiorum* is the production of the hard black sclerotia to which we have referred in the preceding sections. The sclerotium is the resting stage of the fungus, just as the hard bean or the grain of corn is the resting or overwintering stage of these crops. It enables the organism to live through the unfavorable winter months and start a new crop the following summer. The sclerotia, of irregular shapes and sizes, are composed of closely packed white fungous cells with a black exterior rind.

Sclerotia may appear on almost any part of the affected host plant, particularly in the last stages of decay. However, they occur in the greatest abundance in the hollow interior of affected stalks. In Shade tobacco fields they continue to develop even after the stalks are cut down. Examination of these dried stalks on the ground shows great numbers of sclerotia which scatter into the top layers of soil when the field is prepared for the winter. In stalk-cut tobacco, after the leaves are stripped off in the shed, the stalks are drawn out and spread on the field to rot down for fertilizer. The sclerotia from the hollows of the stalks find their way back into the soil when they decay or are broken up with a harrow.

After the sclerotia have rested in or on the ground for several weeks or months, or over winter, structures known as apothecia sprout from them when the weather becomes favorably warm and moist (Figure 9). One to

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[1] Wolf, Frederick A. Gray mold of tobacco. Journ. Agr. Res. **43**:167-175, 1931.

[2] Peters, L. Krankheiten und Beschaedigungen des Tabaks. Mitt. K. Biol. Anst. Land, u. Forstw. **13**:7–76, 1912.

a dozen apothecia develop from a single sclerotium. Each has a very slender stem which may be from a quarter of an inch to an inch or more long. At its apex it expands into a brown vase-like structure (Figure 9) a quarter of an inch across or less. As it matures, the rim turns outward until it recurves. The inner or upper surface of this little goblet (Figure 7B) is lined with closely packed elongated sacs (asci) each of which contains eight spores (Figure 7C). Both the sacs and the spores are too small to be seen with the naked eye.

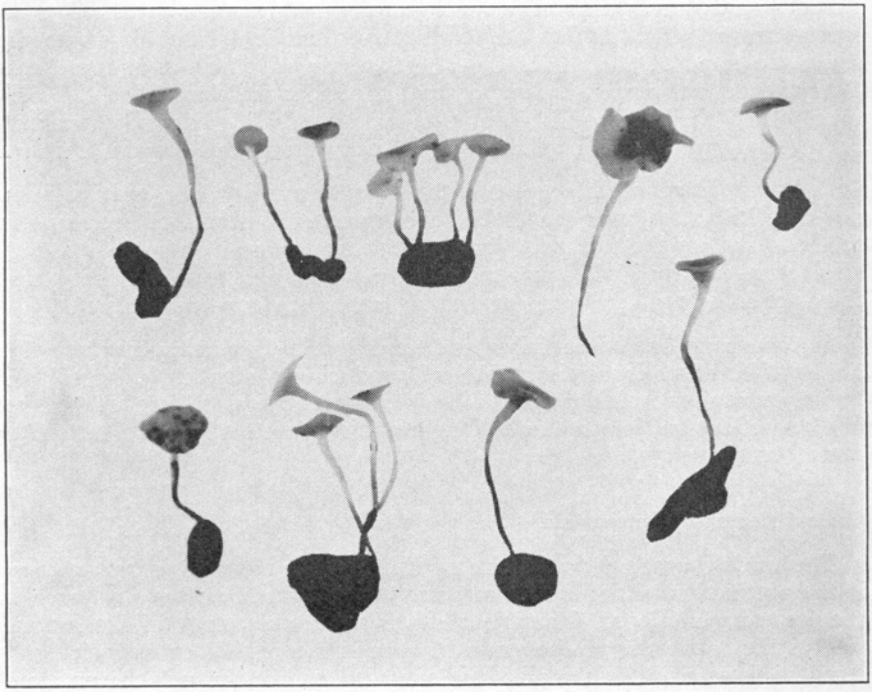

Figure 9. Apothecia developed from sclerotia.

When the spores are mature they are forcibly ejected from the asci into the air where they float around like particles of dust. From a mature apothecium, large numbers of spores are ejected simultaneously in a "puff" which may frequently be seen with the naked eye as a minute white cloud. The spores finally come to rest. If their resting place is damp or if they fall in a drop of water they germinate within two or three hours. In the process of germination a slender tube grows out, branches and divides into cells (Figure 7E). If this occurs in an unfavorable location, the germ-tube dies. But if germination takes place on a favorable spot on the host plant, such as the corolla of the tobacco flower, the germ tube grows through the epidermis and branches in the plant tissues. A toxic substance which it secretes kills the cells of the host. The fungus then continues to live on the decomposition products of the plant cells. When this infected corolla falls on and adheres to a tobacco leaf, the life cycle repeats itself.

Some laboratory studies on life history. In order to study the development of the apothecia from the sclerotia and to more certainly identify the species, a quantity of sclerotia is being treated in each of the following ways:

1. Buried outdoors in sandy soil, very close to the surface. These were planted in October 1942 and will be left undisturbed until next year.

2. Buried in moist sand in a large covered dish and kept on a laboratory bench in strong but diffused light. The laboratory temperature will vary from 60° to 70° F. during the months of this test which began in September 1942.

3. Placed on or slightly under the surface of damp peat moss in a covered dish in the same location as number 2.

4. Placed on water agar slants kept in the same laboratory locations as numbers 2 and 3. This proved to be the simplest way of developing the apothecia under sterile conditions. One per cent of pure agar is dissolved in water, sterilized and slanted in ordinary test tubes. Sclerotia from a pure culture are then transferred with a sterile needle to the surface of the water agar slant. The tubes are kept under a bell jar on the bench to prevent too rapid drying out.

The length of time required for apothecia to appear was about the same for all three methods tried in the laboratory. In about four weeks slender wire-like horns, not over a thirty-second of an inch in diameter, sprouted upward from the surface of the sclerotium. When they were about a half inch high they began to expand at the top and a depression appeared in the apex. The expansion continued until a cup was formed as previously described. At the end of six weeks the spores were mature, white clouds puffing from the apothecia when they were disturbed.

The greatest number of apothecia resulted from the peat moss method. Many of the sclerotia in the moist sand decayed without producing apothecia. Only a small proportion of apothecia sprout at any one time, a fact that has been observed by investigators of this fungus on other kinds of plants. The lot of sclerotia collected in September started to sprout the middle of October and new ones continued every week. Such a continuous succession of spore production guarantees a plentiful supply of spores whenever conditions in the field are right for infection of the new crop.

Ascospores placed in a drop of water on a glass slide began to germinate in two hours at laboratory temperatures. A stout process grew out of one or both ends of the ovate spore. This elongated rapidly, then branched and became septate, as shown in Figure 7E. The short time required for germination and the rapid growth of the germ tube and mycelium help explain the rapidity of Sclerotinia rot under favorable conditions in the field and in the curing shed.

Remedial Measures

The practical grower is naturally most interested in learning how he can avoid or reduce the damage from Sclerotinia and Botrytis diseases. With a better understanding of the fungi involved, their seasonal life history, and particularly the environmental conditions favoring them, we are better able to determine what modifications of our present practices could be expected to reduce the damage. Fortunately, most of the remedial practices that would suggest themselves are already being used and the only improvement would be in their wider adoption and some modifications in timing.

One essential to the occurrence and progress of these diseases is moisture. Every investigator who has worked with Sclerotinia has been impressed with the absolute dependence of the fungus in all its stages on this condition. This furnishes the key to control. The disease can be stopped if the surrounding atmospheric condition can be kept sufficiently dry.

Bed rot, as indicated in the previous discussion, has been pretty well controlled since the first decade of the century by the use of steam sterilization of the soil. Where it does appear, its control calls for the same practices as recommended for other types of bed rot: (1) Beds should be kept well ventilated. This practice prevents saturation of moisture in the air under the glass sash. (2) Thick sowing of seed should be avoided. Close stands of plants mean poor ventilation between seedlings and also make it easier for the fungus to spread from plant to plant. (3) Overwatering should be avoided. Sprinkle beds in the morning so that the leaves will be dry before night. (4) Heavy soils and damp locations should be avoided for seed beds. (5) Spraying the beds often with bordeaux mixture or similar fungicides, starting when the plants are very small, should prove helpful in the control of this as well as other bed rots. Most of these practices are already in use by the majority of growers. They involve little if any extra expense or labor.

For leaf-rot and stalk canker in the Shade fields, no practical remedy has suggested itself. Doubtless both could be eliminated if the tops and blossoming suckers could be removed before the corollas fall. But such a method is not practical because topping and suckering make the upper leaves of tobacco thicker and coarser and therefore not so suitable for wrappers.

The pole rot phase of the disease can be controlled by properly "firing" and ventilating the sheds. If the Shade grower finds many dead-blossom spots on the leaves before they are hung, it is important that "firing" be started as soon as possible. For other types of pole rot a delay of several days may be made after filling the shed, but Sclerotinia and Botrytis rots work best on fresh green leaves, hence the importance of immediate firing as soon as the leaves are hung.

Damage can be avoided or reduced by throwing out the leaves that show dead-blossom spots at the time the leaves are strung on the lath. This is already practiced by some growers.

GIANT BROADLEAF

One of the characteristics of the tobacco species, *Nicotiana Tabacum*, is its tendency to mutate. New forms of plants or mutants, suddenly appear without any apparent reason. Farmers and students of tobacco culture are familiar with mutations, some of which frequently reappear. A well-known example is that of partial chlorosis of leaves, commonly called "marbling." There are various other types of munitions which every grower who studies his plants carefully learns to recognize.

Figure 10. Giant Broadleaf Tobacco.

One of the less frequent mutations, which has been observed and recorded in a number of varieties of tobacco, is a form that we may call "giant tobacco." Instead of developing a cluster of flowers at the top during the latter part of the growing season, the plant continues to produce leaves and elongate the stalk indefinitely throughout the entire growing season. In this way it may produce three or four times as many leaves as a normal plant, but no seed. Many growers of Shade tobacco have observed such plants in their fields. We have sometimes brought these Shade plants into the greenhouse where they grow to be 10 to 15 feet high with 75 to 100 leaves. They finally blossom and seed in January or later. When the seed is planted it produces a generation of plants, all of which have the same giant habit. Another example of giant tobacco is a commercial variety grown in Maryland, called Maryland Mammoth. Since it will not seed in that climate, growers have the seed grown for them in southern Florida.

It is likely that all commercial varieties of tobacco have a latent capacity to produce giant mutants, although we have never found or heard of such an occurrence in Broadleaf or Havana Seed varieties. Late in the 1942 season, however, we were asked to visit the farm of D. E. and R. C. Neelans in Hazardville to see what they thought was a new variety of tobacco which had suddenly appeared in their Broadleaf fields. There were many plants, probably one in every two or three hundred, which did not set seed but continued to elongate at the tip until they had some 70 leaves at the time we saw them. Some of these, which they had left in the field after harvesting the rest of the crop, are shown in Figure 10. The plants produced no suckers unless they were topped. This was a typical case of gigantism. One of the plants was taken into the Station greenhouse to see if it would set seed during the winter. It began to blossom in January.

The Neelan brothers had never before observed this phenomenon in their tobacco. The seed came from a neighboring farmer who also assured us he had never seen giants in his tobacco.

The question immediately arises as to whether a plant with so many leaves would have added commercial value. Experiments to answer this question have not been made. The growers had harvested a few of the giant plants but found they were unwieldy to handle and cured out slowly.

STUDIES ON BLACK TOBACCO

II. Field Crop Response to Phosphate and Lime Fertilization

STUART B. LECOMPTE, JR.

"Black" tobacco is a term used by handlers of Shade tobacco to designate an inferior grade of leaves which have cured to an objectionable dull black. In Bulletin 444 of this Station it was reported that such tobacco came from fields which were excessively acid and deficient in phosphorus and calcium (7).[1] Per unit weight of dry matter, black leaf generally contained notably more manganese and iron than light, good quality leaf. It was assumed that excessive absorption of these metals and development of the black pigment in the curing process bore some interrelationship though not necessarily one of cause and effect.

The application of phosphates or lime is the well-known remedy for soil where excessive soluble iron, manganese and aluminum compounds exert unfavorable effects on plant growth (2, 6, 15). Therefore experiments were planned to yield evidence on the relation of black tobacco to phosphate and lime fertilization.[2]

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[1] Numbers in parentheses refer to list of publications on p. 154.

[2] The writer gratefully acknowledges helpful advice by Dr. M. F. Morgan, Agronomist in Charge, Connecticut Agricultural Experiment Station, New Haven, Conn., in regard to fertilization.

During 1941 and 1942 studies were made of the effects of fertilization with 48 per cent superphosphate and hydrated lime upon quality, value, yield and plant development of field-grown Shade tobacco.

Experimental Fields

Two pieces of land, designated hereafter as Field A and Field B, were treated experimentally in 1941. These were located about 10 miles apart and were each nearly an acre in size. In 1942 only the experiment on Field A was continued.

Field A was a Merrimac fine sandy loam (13) which had produced black tobacco in 1939 and 1940. Tests on May 1, 1941, showed that the soil was strongly acid with low available phosphorus, very low calcium, medium magnesium supplies and very high amounts of active aluminum. The quality of leaf grown on several acres of this land was so poor in 1939 and 1940 that tobacco culture was discontinued on the tract. Although the field was part of a large Shade plantation it was the only area on the farms where black leaf had been a serious problem. It had lain fallow from 1924 to 1939. The tobacco fertilizer broadcast in 1939 and 1940 had a basicity equivalent to about 50 to 100 pounds of limestone per acre per year and supplied nutrients approximately at the following annual rate in pounds per acre: nitrogen, 230; phosphoric acid, 96; potash, 211; calcium oxide, 65 and magnesium oxide, 77. The low equivalent basicity of this treatment probably had little or no effect in neutralizing soil acidity because experience has shown that about a ton of calcium carbonate per acre is needed to decrease the acidity of this type of soil one pH unit.

Field B was an eroded Merrimac sandy loam with considerable gravel. Prior to 1938 it had been woodland. In 1938 it was planted to potatoes and in 1939, 1940 and 1941, to tobacco. The tobacco crop from Field B was very inferior in 1939 and 1940 because of the large proportion of black-curing leaves. Soil tests (14) from 1938 to 1940 showing strong acidity, very low phosphorus and calcium, were as follows:

		NO_3–N	NH_3–N	P	K	Ca	Mg	Al	Mn
	pH	Pounds per acre to plow depth							
Fall 1938	5.12	40	10	100	500	400	50	—	—
Fall 1939	4.80	100	10	25	600	400	100	—	—
June 1940	4.08	100	10	10	300	400	50	—	—
April 1941[1]	4.82–5.72	2–100	10	15	150–600	400	60–100	35–400	2

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[1] Sampled prior to fertilization.

Nutrients added in pounds per acre, 1939 to 1941, were as follows:

	N	P_2O_5	K_2O	CaO	MgO
1939	272	304	286	417	196
1940	199	329	197	902	171
1941	192	212	195	578	42

The basic effect of the fertilizer program was equivalent to the addition of calcium carbonate at a rate per acre of approximately 360 pounds in 1939, and 996 pounds yearly in 1940 and 1941.

In the spring of 1941 an experimental tract on Field A, 11 bents long and three bents wide, was tented, cultivated as usual and fertilized uniformly by spreader with a mixture of the same composition as used in the previous two years. Nine test treatments of granular superphosphate (total phosphoric acid 48.0 per cent; available, 47.7 per cent) and hydrated lime (calcium oxide 61.3 per cent, magnesium oxide 3.1 per cent) were set up as follows, amounts stated in pounds per acre:

	Treatment	
No.	Superphosphate	Lime
1	0	0
2	200	0
3	400	0
4	0	1,000
5	200	1,000
6	400	1,000
7	0	2,000
8	200	2,000
9	400	2,000

Treatments were arranged in randomized blocks and each plot conformed to a bent of Shade tobacco, or one-fortieth acre. Each treatment was in triplicate. Extra treatments needed to complete the 33-bent field were so chosen that treatment Number 3 was repeated five times, treatment Number 6, six times and treatment Number 9, four times. On May 27 and 28 the fertilizer test materials were broadcast by hand and the tract was disc-harrowed. On June 3 the plots were set to Shade tobacco of the same genetic strain which in the two years previous had grown on this soil and had cured black.

In the spring of 1941, 16 plots on Field B, each one bent wide and two bents long, were laid out serially side by side, uniformly cultivated and fertilized as indicated above. Five test treatments of granular superphosphate (total phosphoric acid 46.8 per cent; available, 46.6 per cent) and hydrated lime (calcium oxide 71.0 per cent, magnesium oxide 0.6 per cent) were arranged systematically in three adjacent blocks. Each treatment was repeated once in each block. The check treatment, Number 1, was repeated once in addition, making four replications for this treatment only. Identified by the same treatment numbers used for Field A, the amounts of material broadcast on Field B were the same as for treatment numbers in Field A: 1, 2, 3, 5 and 6. Dates of fertilizing and setting tobacco on Field B were about the same as for Field A in 1941.

The plan and execution of the experiment on Field A in 1942 were the same as in 1941 except for the following details. No cover crop was used for the winter of 1941-42. No lime was applied to any plot in 1942. All plots which received 48 per cent superphosphate in 1941, either with or without lime, were treated in 1942 with a similar superphosphate at the same rates per acre as in 1941. The material used in 1942 contained 46.8 per cent available, 47.2 per cent total phosphorus pentoxide and 2.41 per cent fluorine as analyzed by Dr. E. M. Bailey, Station Chemist. The 1942 superphosphate was broadcast by hand on May 11 and harrowed May 12. The tobacco fertilizer, applied uniformly about two weeks later over the whole test area by spreader, supplied nutrients in pounds per acre thus: nitrogen, 190; phosphorus pentoxide, 115; potassium oxide, 245; calcium oxide, 129 and magnesium oxide, 77. The experimental area was set to Shade tobacco on May 29.

Soil samples in 1941 were taken with an auger to 6-inch depth in 10 borings spaced uniformly over each bent. This was done before application of any fertilizer, again while the crop was growing in July and, finally, after the fourth picking in September. The borings from each bent were pooled and thoroughly mixed. Complete soil tests were made on the sample from each bent. Soil test data of July and September from treatments made in triplicate were averaged and are presented in Table 4.

Soil samples of 1942 consisted in four auger borings to 6-inch depth taken from each half, the east and west respectively, of each plot of Field A. These were taken, first, before any fertilizer applications; second, in July two days before the first picking and, finally, in late August after harvest. Soil test data for July and August 1942 are presented in Table 4 as averages of 12 samples representing three plots of a single fertilizer treatment.

Observations on Growth

On Field A, during the 1941 growing season and at picking time, there were striking differences in growth of tobacco among the plots. Plants on check, i.e., untreated, plots showed the slow growth, dark green leaf color and narrow leaf base described as typical of phosphorus deficiency by McMurtrey (11, 12) and by Anderson, Swanback and Street (3). The plants were retarded in flowering and their leaves appeared more erect and less spreading, and felt thicker and heavier than leaves from phosphated plots. In the early stages of growth a bronzing was noted on the lower leaves of certain stunted plants. A similar coloration is reported by Anderson, Swanback and Street (3) as a symptom of phosphorus deficiency. No other conspicuous abnormalities marked the crops on untreated plots.

No striking contrast in growth, but a general vigorous condition, was noted among the plots of Field B.

Harvesting, Curing and Fermentation

Uniform cultivation, budding, harvesting, curing and fermentation of the tobacco were practiced in the usual manner (1). In 1941 four pickings on Field A were taken as the leaves matured: first, leaves number 1, 2 and 3 (counting from base of plant) on July 28; second, leaves 4, 5 and 6 on August 13; third, leaves 7, 8, 9 and 10 on August 29; and fourth, leaves 11, 12, 13 and 14 on September 6.

In 1942 three pickings of three leaves each were taken on Field A, the first on July 22, the second on July 29 and the third on August 6.

TABLE 4. SOIL TEST AVERAGES IN RELATION TO FERTILIZER TREATMENT, FIELD A, 1941 AND 1942, AND FIELD B, 1941. ANALYSIS BY THE UNIVERSAL SOIL TESTING SYSTEM

	Treatment	Pounds per acre			Concentration of active constituents Pounds per acre to plow depth								Ratio	
Field	No.	48 per cent Super-phosphate	Hydrated lime	Acidity pH	NO_3–N	P	K	Ca	Mg	Al	Mn	Fe	P/Al	PxCa/Al
A, 1941[1]	1	0	0	4.99	77	47	500	542	183	300	12.5	20	0.16	85
	2	200	0	5.11	70	63	500	554	179	304	10.2	21	0.21	115
	3	400	0	5.08	70	63	500	675	167	317	7.8	22	0.20	134
	4	0	1,000	5.43	72	55	500	1,458	167	225	5.0	18	0.24	356
	5	200	1,000	5.42	85	53	500	1,271	179	221	7.0	18	0.24	305
	6	400	1,000	5.53	82	74	500	1,513	175	203	5.0	18	0.36	551
	7	0	2,000	5.87	77	54	500	1,733	175	136	2.5	17	0.40	688
	8	200	2,000	5.90	75	67	500	1,700	208	140	3.0	16	0.48	814
	9	400	2,000	5.99	67	94	500	1,792	183	153	3.0	15	0.61	1,101
A, 1942	1	0[2]	0[2]	4.84	30	62	458	483	60	277	3	—	0.22	108
	2	400	0	4.78	18	60	321	488	55	181	3	—	0.33	162
	3	800	0	4.90	27	70	417	546	64	240	3	—	0.29	159
	4	0	1,000	5.07	34	44	454	583	65	173	2	—	0.25	148
	5	400	1,000	5.03	33	59	375	700	57	206	2.5	—	0.29	200
	6	800	1,000	5.11	22	71	367	688	60	138	3	—	0.51	354
	7	0	2,000	5.34	30	57	442	738	68	167	2	—	0.34	252
	8	400	2,000	5.28	36	62	388	712	62	123	2	—	0.50	359
	9	800	2,000	5.24	25	69	396	817	66	173	2	—	0.40	326
B, 1941[1]	1	0	0	5.34	63	67	500	1,656	206	202	8.4	24	0.33	549
	2	200	0	5.45	55	92	500	1,658	188	197	5.3	21	0.47	774
	3	400	0	5.38	78	98	500	1,517	150	149	7.3	20	0.66	998
	5	200	1,000	5.85	69	108	500	2,167	146	88	2.5	18	1.23	2,660
	6	400	1,000	6.16	70	154	500	2,417	163	113	2.7	18	1.36	3,294

[1] Analysis by Dr. M. F. Morgan, Connecticut Agricultural Experiment Station.
[2] Total application for two-year period, treatments 1 to 9.

On Field B in 1941 four pickings of three leaves each were made, the first on July 16, the second on August 4, the third about August 13 and the fourth on August 22.

Only plants of the central eight rows of a bent were picked for data, no plant being used which grew within less than 3 feet of the plot boundary. In any bent, care was exercised to prime the same group of plants successively through all pickings. Generally at least 400 leaves were harvested per plot at each picking. The crop from each bent was tagged, strung on lath and air-cured in the same section of the shed with other leaves from test plots picked on the same day.

In 1941 tobacco from one check plot of Field A (Number 11 of Tables 10 to 12) was observed to cure much more slowly, i.e., retained green pigments for a longer time, than that from two phosphate-treated plots (Numbers 19 and 20, Tables 10 to 12), the first unlimed, the second limed.

When curing was complete, all leaves from one picking were bulked and fermented together with leaves of the same picking from other fields. Some black tobacco was evident throughout the harvest from Field A as each priming approached completion of the curing process in the shed. However, leaf color and quality were quantitatively estimated by professional sorters only after the test tobacco had been properly fermented.

Sorting Methods

Effectiveness of the fertilizer treatments was judged by four criteria: leaf quality, value, yield and stalk growth. Other criteria, used only in 1942 were: proportion of flowering plants and plant height at picking time and average length of fermented leaves. Quality was estimated by the commercial Shade-leaf grading system in 1941. In 1942 all leaves lighter than V grade were defined as light and arbitrarily assigned the value of YL2 grade. All leaves of V grade or darker were defined as black and arbitrarily assigned the value of KVB grade. Value was computed from a U. S. Department of Agriculture listing of minimal prices standardized among the tobacco growers of the Connecticut Valley for 1941. Yield (one index of growth) was measured by the sorted weight of the 400 sample leaves picked in each priming from each plot. Leaves of the 1941 crop on both fields were sorted only for quality and not for length. Values of the 1941 crop were therefore determined by assuming that all leaves of the first picking were 11 inches long and by assigning the standard minimal price for that length to the grades that appeared. Similarly it was assumed that all leaves from other pickings were 12 inches long. Although the 11 and 12-inch lengths were chosen to represent roughly the leaves as sorted, in fact shorter lengths were noted from bents with poorer growth and lower crop weight. Conversely, longer leaves than the assumed standard were found where yield was greater.

The average leaf length of the 1942 crop, Field A, was ascertained for each plot and priming. Values were fixed by the same standard listing employed in 1941. The quality grades were only two in 1942, light and black, as previously stated, and the values for these grades were arbitrarily

TABLE 5. QUALITY OF SHADE TOBACCO AS INDICATED BY PERCENTAGES OF DARK GRADES (V, V2, KV, KV2, AND KVB), BLACK GRADES (KV2 AND KVB), PHOSPHATE AND LIME FERTILIZATION, SOIL ACIDITY AND SOIL PHOSPHORUS/ALUMINUM RATIO ON FIELD A, 1941 AND 1942, AND ON FIELD B, 1941

Field	Treatment	Pounds per acre		Percentage of sorted leaf weight — Leaf order of picking from base of plant								Soil	
		48 per cent		1 to 3		4 to 6		7 to 10		11 to 14			
Field	No.	Super-phosphate	Hydrated lime	Dark	Black	Dark	Black	Dark	Black	Dark	Black	pH	P/Al ratio
A, 1941	1	0	0	74.9	66.9	49.7	17.8	50.1	9.6	53.9	18.3	4.99	0.16
	2	200	0	68.6	57.2	36.4	11.0	34.7	4.6	31.1	7.1	5.11	0.21
	3	400	0	64.2	50.9	41.4	16.0	48.1	17.5	39.8	13.0	5.08	0.20
	4	0	1,000	79.3	66.0	51.9	16.8	51.0	16.7	51.4	23.1	5.43	0.24
	5	200	1,000	74.7	59.0	41.2	13.3	47.2	16.3	59.9	27.4	5.42	0.24
	6	400	1,000	60.6	46.1	46.4	8.5	43.6	11.1	34.4	7.1	5.53	0.36
	7	0	2,000	72.3	60.1	34.0	9.0	46.6	11.8	47.8	15.0	5.87	0.40
	8	200	2,000	77.3	63.9	38.5	8.8	49.7	15.4	42.0	11.4	5.90	0.48
	9	400	2,000	42.9	30.8	26.9	5.3	41.0	8.8	45.4	12.2	5.99	0.61
				1 to 3		4 to 6		7 to 9		10 to 12			
A, 1942	1	0[1]	0[1]	38.2		15.4		1.8		—		4.84	0.22
	2	400	0	15.9		1.8		0.6		—		4.78	0.33
	3	800	0	5.9		0.1		0.0		—		4.90	0.29
	4	0	1,000	9.7		1.8		0.0		—		5.07	0.25
	5	400	1,000	10.4		0.8		0.0		—		5.03	0.29
	6	800	1,000	2.1		0.7		0.0		—		5.11	0.51
	7	0	2,000	3.6		1.2		0.0		—		5.34	0.34
	8	400	2,000	1.0		3.7		0.0		—		5.28	0.50
	9	800	2,000	1.3		0.3		0.0		—		5.24	0.40
				Dark	Black	Dark	Black	Dark	Black	Dark	Black		
B, 1941	1	0	0	44.7	17.9	23.4	6.0	23.4	1.6	12.4	0.0	5.34	0.33
	2	200	0	37.8	12.8	21.4	4.7	20.8	3.2	6.5	0.0	5.45	0.47
	3	400	0	34.9	17.1	38.0	18.2	30.9	0.8	14.8	0.0	5.38	0.66
	5	200	1,000	38.8	17.9	34.4	12.8	13.6	0.0	11.8	0.4	5.85	1.23
	6	400	1,000	30.6	10.5	30.4	9.0	23.6	2.4	13.2	0.0	6.16	1.36

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[1] Total application for two-year period, treatments 1 to 9.

uniform for all plots. Hence, no just comparison of value can be made between the years 1941 and 1942. Nor are the values for either year commercially correct since length was assumed the first year and grade values assumed in the second. However, the figures of value do allow rough comparisons between plot treatments for one field in one year.

Experimental Results

Quality

Table 5 shows the proportion of all dark grades as per cent of sorted weight for triplicate treatments in each priming. Dark grades are here considered to be the V and KV classes, i.e., V, V2, KV, KV2 and KVB. Leaves of KV2 and KVB grades were identified as "black" tobacco by experienced tobacco workers. Until the term "black" is better defined, the judgment of such tobacco experts must be the standard of reference.

Under the conditions of the experiment, Table 5, Field A, 1941, indicates the following points:

1. The proportion of black and dark grades in each picking was considerable but was especially great in the first picking, i.e., the three leaves lowest on the plant.

2. For the lowest six leaves, untreated check plots gave the highest percentage of dark and black grades, showed the most acid soil (average pH 4.99), highest active soil manganese (Table 4) and the lowest soil phosphorus/aluminum ratio (0.16). Conversely, for the lowest six leaves, the lowest percentage of dark and black leaves was in the heaviest treatment of phosphate and lime where soil showed the least acidity (pH 5.99), lowest active manganese and the highest phosphorus/aluminum ratio (0.61).

3. In leaves 7 to 14 the lowest percentage of darks and blacks occurred with the application of 200 pounds per acre of superphosphate, and no lime.

4. The percentage of dark and black leaves from treatments 4 to 8, given a half ton and a ton of lime per acre, was somewhat greater or only slightly smaller than percentages of similar grades on unlimed plots in the first two pickings (leaves 1 to 6). Statistical analysis of variance,[1] not presented in this paper, on the relation of KV2 and KVB grade percentages of the first picking to fertilizer treatments in 1941 has shown that lime had no significant reducing effect but even tended to increase the proportion of black tobacco in the basal three leaves, whereas phosphate exerted a pronounced effect in lightening the color of the crop.

5. Only treatments Numbers 3, 6 and 9, with highest rate of phosphate (400 pounds per acre), show systematic decrease in per cent of KV2 and KVB in the first three pickings in relation to increased lime. The same may be said of all dark grades, V's and KV's, with the exception of treatment Number 6, second picking.

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[1] Thanks are due Dr. C. I. Bliss, Biometrician, Connecticut Agricultural Experiment Station, New Haven, for helpful guidance in computation.

6. In leaves 1 to 6, plots with phosphate had a smaller proportion of dark and black grades than plots with no phosphate. The percentage of such grades in leaves 1 to 3 was less with increased phosphate, irrespective of lime treatments, with the exception of treatment Number 8. In this exceptional case perhaps the abundant lime application (1 ton per acre) temporarily reduced the phosphate availability. This may have been an example of temporary overliming injury due to disturbed phosphate nutrition as described by Pierre (17), Pierre and Browning (18), or an example of extensive formation in the soil of poorly available calcium fluorphosphate as studied by MacIntire (9), MacIntire and Hatcher (10).

7. In the first two pickings where the proportion of dark leaves was greatest there was less dark and black tobacco as phosphate was increased from 0 to 400 pounds per acre.

Table 5 shows that on Field A in 1942 the greatest proportion of black tobacco was derived in any picking from untreated check plots. There was much less black tobacco in the second picking than in the first, and there was practically no dark or black tobacco above the seventh node. The trend toward reduced proportion of black leaf with heavier application of both phosphate and lime is striking in the first picking. The same trend is apparent in the second picking except in treatment Number 8; since the differences in the percentage of black tobacco are small for leaves 4 to 6 where the lime of 1941 was applied (treatments 4 to 9) this trend is mainly significant for the contrast between treated and untreated plots. The greatly reduced yield of black and dark tobacco for all plots in 1942 contrasted with 1941 is interesting. Perhaps not only weather but the ameliorative effect of continued annual application in the general tobacco fertilizer mixture of phosphorus pentoxide at a rate of 96 to 115 pounds per acre, calcium oxide at 129 pounds per acre, and magnesium oxide at 77 pounds per acre played roles in reducing the yield of black leaf.

Results in 1941 from different plot treatments on Field B showed weak contrasts. This was perhaps the effect of considerable phosphate and lime fertilization prior to the experiment.

Table 5 shows that the check treatment of Field B in 1941 produced the highest proportion of all dark grades in the first picking and that least dark grades were produced under treatment Number 6 with 400 pounds per acre of superphosphate and 1,000 pounds per acre of hydrated lime. In higher pickings from Field B, however, this trend is not apparent and differences among the plots may be insignificant.

Field B grew much less dark tobacco in 1941 than Field A. This suggests superior fertility in Field B. The soil phosphorus/aluminum ratio of Field B was higher than in Field A (Table 4).

Value

Money values of the test crops in relation to treatment are given in Table 6.

TABLE 6. MONEY VALUE OF SHADE TOBACCO IN RELATION TO PHOSPHATE AND LIME TREATMENT, FIELD A, 1941 AND 1942, AND FIELD B, 1941

	Treatment, pounds per acre			Value of 400 leaves Leaf order of picking from base of plant					
Field	No.	48 per cent Superphosphate	Hydrated lime	1 to 3	4 to 6	7 to 10	11 to 14	Total value 1,600 leaves	Value, per pound 1,600 leaves
A, 1941[1]	1	0	0	$0.55	$3.61	$5.33	$4.78	$14.27	$1.60
	2	200	0	0.94	5.20	6.35	5.86	18.35	1.90
	3	400	0	1.10	5.29	5.62	6.35	18.36	1.81
	4	0	1,000	0.73	4.26	5.04	5.06	15.09	1.60
	5	200	1,000	0.89	4.71	5.11	4.36	15.07	1.54
	6	400	1,000	1.06	4.75	4.32	5.58	15.71	1.62
	7	0	2,000	0.73	4.15	4.59	4.98	14.45	1.63
	8	200	2,000	0.75	4.09	4.44	5.61	14.89	1.61
	9	400	2,000	1.30	5.18	5.27	5.16	16.91	1.74
				1 to 3	4 to 6	7 to 9	10 to 12	Total value 1,200 leaves	Value, per lb. 1,200 leaves
A, 1942[2]	1	0[3]	0[3]	0.47	1.84	3.33	—	5.64	1.14
	2	400	0	0.84	2.88	3.71	—	7.43	1.29
	3	800	0	1.41	3.04	3.80	—	8.24	1.38
	4	0	1,000	0.52	2.17	3.24	—	5.94	1.19
	5	400	1,000	0.88	2.76	3.52	—	7.16	1.30
	6	800	1,000	1.03	2.75	3.54	—	7.32	1.33
	7	0	2,000	0.39	1.59	2.60	—	4.58	0.98
	8	400	2,000	0.85	2.13	3.27	—	6.25	1.24
	9	800	2,000	1.11	2.86	3.51	—	7.49	1.35
				1 to 3	4 to 6	7 to 9	10 to 12	Total value 1,600 leaves	Value, per lb. 1,600 leaves
B, 1941[1]	1	0	0	1.28	4.86	6.36	6.39	18.89	2.18
	2	200	0	1.59	5.51	6.63	6.87	20.60	2.27
	3	400	0	1.61	4.38	6.37	5.82	18.18	2.06
	5	200	1,000	1.57	4.85	6.42	5.61	18.45	2.08
	6	400	1,000	1.60	4.66	6.60	6.89	19.75	2.36

[1] Values based on assumed length, actual grades. [2] Values based on actual length, assumed grades. [3] Total application for two-year period, treatments 1 to 9.

The total 1941 crop value, Field A, per 1,600 leaves, value per 400 leaves in each picking and crop value per pound were least with check plots and greatest with the application of 200 pounds per acre of 48 per cent superphosphate alone. This rate of phosphate achieved a total value for 1,600 leaves 28.7 per cent higher than the check plots. The value per pound of the whole crop from treatment with 200 pounds per acre of superphosphate was 18.8 per cent greater than the value per pound of crops with no treatment. The deleterious effect of lime is shown in the lower values of limed and phosphated plots contrasted with the value of plots treated with phosphate alone. Lime alone enhanced only slightly the value of the crop per pound. Lime alone at the half-ton rate per acre generally gave, for the first, second and fourth pickings, a value slightly greater than that for check plots, but a ton-per-acre rate of lime produced either the same effect as achieved with the half-ton rate or was even less beneficial.

Values of the 1942 crop, shown in Table 6, were highest per leaf and per pound in each picking for plots treated most heavily with phosphate alone, i.e., 800 total pounds per acre of 47 to 48 per cent superphosphate in two years. The total value of 1,200 leaves picked on plots with this treatment was 46.1 per cent higher than the value of an equal number of leaves from check plots. Similarly, value per pound was 21.1 per cent higher on treatment Number 3 than on treatment Number 1, the check.

Under the conditions of this experiment, liming the soil was not profitable although effective in reducing the yield of black tobacco. A ton of hydrated lime per acre applied in 1941 produced in the 1942 crop a percentage of black tobacco slightly smaller than the percentage of such tobacco produced by a total of 800 pounds per acre of 47 to 48 per cent superphosphate applied in the same two-year period. However, the poor growth resulting on plots limed at the rate of a ton per acre in 1941 gave in 1942 a total value for 1,200 leaves (three pickings) nearly 19 per cent less than that from untreated, check plots. Half a ton of lime alone per acre applied in 1941 gave in the second year a value for 1,200 leaves only 5.3 per cent above the value for untreated plots. For any rate of liming tested on Field A, heavier applications of phosphate resulted generally in greater crop value in both 1941 and 1942.

Values from the 1941 crop of Field B were higher in the first picking as phosphate was applied more heavily. This trend is not seen in other pickings and differences among values for the several treatments are of dubious significance. However, values of Field B are generally superior to values from Field A, 1941. Even the check plots of Field B showed an average value for 1,600 leaves in four pickings 2.9 per cent higher than the top values obtained from any treatment on Field A, 1941.

Growth

Growth relationships are indicated in Tables 7 to 12.

Yield of leaf. From Field A, the heaviest yield of 1941 and 1942 leaf was outstanding for treatment with 400 pounds per acre superphosphate per year, as shown in Table 7. The lowest weight crop grew on plots treated with a ton per acre of lime alone. It is probable that leaf area

Table 7. Weight of Shade Tobacco in Relation to Phosphate and Lime Treatment, Field A, 1941 and 1942, and Field B, 1941

Field	No.	Treatment Pounds per acre		Weight, in ounces, of 400 leaves, shop condition Leaf order of picking from base of plant				Weight, in ounces, of 1,200 leaves, first three pickings	Weight, in ounces, of 1,600 leaves, four pickings	Weight, in ounces, of fresh stalk basal meter (39.37 inches)
		48 per cent Superphosphate	Hydrated lime	1 to 3	4 to 6	7 to 10	11 to 14			
A, 1941	1	0	0	18.2	33.9	45.4	45.3	97.5	142.8	9.8
	2	200	0	24.0	40.1	46.2	44.5	110.3	154.8	10.7
	3	400	0	25.4	41.5	48.4	47.7	115.3	163.0	12.2
	4	0	1,000	21.4	36.6	45.7	47.6	103.7	151.3	11.1
	5	200	1,000	23.4	39.7	46.5	46.5	109.6	156.1	12.2
	6	400	1,000	25.2	38.7	45.9	46.0	109.8	155.8	11.6
	7	0	2,000	20.1	34.6	43.1	43.4	97.8	141.2	11.1
	8	200	2,000	22.6	37.3	43.1	45.2	103.0	148.2	11.1
	9	400	2,000	24.8	39.1	46.2	45.5	110.1	155.6	11.8
				1 to 3	4 to 6	7 to 9	10 to 12			
A, 1942	1	0[1]	0[1]	17.4	26.1	36.2	—	79.7	—	8.6
	2	400	0	21.2	31.2	39.8	—	92.1	—	9.5
	3	800	0	22.7	32.1	40.5	—	95.2	—	10.1
	4	0	1,000	18.2	26.0	34.6	—	78.8	—	9.3
	5	400	1,000	20.6	29.6	37.6	—	87.8	—	8.9
	6	800	1,000	20.7	29.6	37.8	—	88.0	—	8.7
	7	0	2,000	15.7	24.1	33.5	—	73.3	—	7.2
	8	400	2,000	19.4	25.8	34.9	—	80.1	—	8.1
	9	800	2,000	20.6	30.6	37.5	—	88.6	—	8.8
B, 1941	1	0	0	21.0	27.5	42.3	48.1	90.8	138.9	12.4
	2	200	0	22.4	30.5	43.3	49.2	96.2	145.4	13.5
	3	400	0	21.4	30.1	43.0	47.0	94.5	141.5	13.1
	5	200	1,000	21.9	30.2	42.1	47.9	94.2	142.1	13.1
	6	400	1,000	20.8	28.5	29.2	45.3	88.5	133.8	12.1

[2] Total application for two-year period, treatments 1 to 9.

rather than specific gravity of leaf tissue was the dominant factor in determining leaf weights although both area and specific gravity are naturally involved. The treatment with highest phosphate alone gave the heaviest yield in each of the four pickings. Moisture contents of the leaves when weighed immediately after sorting were about 20 per cent of the sorted weight. It is noteworthy that the heaviest tobacco stalks were from plots with the same high phosphate treatment that yielded the greatest weight of leaf.

Leaf weights among different treatments of Field B, 1941, are all more or less alike. Treatment Number 2 (200 pounds per acre superphosphate) which showed the heaviest total yield of leaf and stalk also had the highest crop value.

Stalk growth. Stalk growth was estimated by the fresh weight and the number of leaf nodes of the basal meter length (39.37 inches) of 10 stalks cut at random from each plot a few days after the fourth picking. Average distances between leaf nodes were obtained from the total leaf scars found on the stalks.

Growth of the stalk, judged from average internode length (shown in Table 8), for Field A, 1941, was greatest on plots treated with 400 pounds per acre of 48 per cent superphosphate and one ton per acre of lime. How-

TABLE 8. AVERAGE DISTANCE BETWEEN LEAF NODES AND AVERAGE NUMBER OF LEAVES ON THE BASAL METER (39.37 INCHES) OF STALK, AFTER HARVEST, IN RELATION TO FERTILIZATION, FIELD A, 1941 AND 1942. AVERAGE LEAF LENGTH, FIELD A, 1942

Field	Treatment			Internode average length, in inches	Number of leaves in basal meter of stalk	Average length of sorted leaves in inches		
	No.	Pounds per acre 48 per cent Super-phosphate	Pounds per acre Hydrated lime			Leaf order of picking 1 to 3	4 to 6	7 to 9
A, 1941[1]	1	0	0	2.56	15.4	—	—	—
	2	200	0	2.64	15.0			
	3	400	0	2.75	14.3			
	4	0	1,000	2.48	15.9	—	—	—
	5	200	1,000	2.71	14.5			
	6	400	1,000	2.69	14.6			
	7	0	2,000	2.51	15.7	—	—	—
	8	200	2,000	2.66	14.8			
	9	400	2,000	2.79	14.1			
A, 1942[2]	1	0[3]	0[3]	2.77	14.3	9	12	14
	2	400	0	3.04	13.0	10	13	15
	3	800	0	2.92	13.5	11	14	15
	4	0	1,000	2.85	13.9	9	12	14
	5	400	1,000	2.87	13.7	10	13	14
	6	800	1,000	2.94	13.4	11	13	14
	7	0	2,000	2.88	13.7	8	11	13
	8	400	2,000	2.90	13.6	10	12	14
	9	800	2,000	2.98	13.2	11	13	14

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[1] Measurements show growth attained 95 days after setting.

[2] Measurements show growth attained 89 days after setting.

[3] Total application for two-year period, treatments 1 to 9.

ever, internodes were nearly as long on plots treated with 400 pounds per acre of superphosphate alone. Internodes were shortest on plots which received a half ton per acre of lime but no phosphate and were not much longer where a full ton per acre was applied. Indeed, check plots grew stalks with internodes slightly longer than stalks from plots treated with lime alone. As lime treatment was constant on Field A, 1941-42, and applied phosphate was greater, there were often fewer leaves on the basal meter (39.37 inches) of stalk, or, in other words, the leaf internodes were longer. As phosphate was constant and lime was greater, there was no similar systematic relationship apparent, except in 1942, when treatments 1, 4, 7 and 3, 6, 9 showed a trend to longer internodes with greater lime treatment.

On Field A in 1942 the longest internodes were found in treatment Number 2 where a total of 400 pounds per acre superphosphate was applied in the two-year period; the shortest internodes were again found on check plots. Though differences in average internode length were small and perhaps of doubtful significance it may be noted that the average internode was longer on plots treated with some superphosphate than on plots receiving no superphosphate. Leaves were longer where phosphate was applied than they were elsewhere on Field A, 1942 (Table 8).

Stalk internode length on Field B was similar among the five treatments in 1941, and averaged 2.91 inches.

Flowering and plant height. In 1942 at picking times on Field A, the average plant height and percentage of plants with open flowers were roughly estimated in each bent. Data from these observations are shown in relation to cumulative treatments of 1941 and 1942 in Table 9. Poor plants with tardy flowering grew on plots which were untreated or were treated with lime only, in contrast to the plant response on phosphated plots. Delayed plant maturity was to be expected on phosphorus-deficient soil (2).

TABLE 9. AVERAGE PLANT HEIGHT AND PERCENTAGE OF PLANTS FLOWERING, AT PICKING TIMES, IN RELATION TO FERTILIZATION OF SHADE TOBACCO, FIELD A, 1942

Treatment Pounds per acre			Average plant height in feet			Percentage of plants flowering on		
No.	48 per cent Super-phosphate	Hydrated lime	July 22 First picking	July 29 Second picking	August 6 Third picking	July 22 First picking	July 29 Second picking	August 6 Third picking
1	0[1]	0[1]	2.8	3.8	4.9	0	15	33
2	400	0	4.9	5.3	6.5	0	33	100
3	800	0	5.6	6.1	7.2	0	100	100
4	0	1,000	3.3	4.8	5.9	0	33	75
5	400	1,000	5.3	5.4	6.3	0	66	75
6	800	1,000	5.3	6.0	7.0	0	100	100
7	0	2,000	3.2	4.0	5.3	0	0	50
8	400	2,000	4.5	4.7	6.2	0	50	100
9	800	2,000	5.3	6.0	7.5	0	100	100

[1] Total application for two-year period, treatments 1 to 9.

Area and weight of fresh leaves. Leaf growth comparisons are given for three plots of Field A, 1941, in Tables 10 to 12. Ten to 20 leaves were picked at random through the bent, but at the proper picking level on any stalk, at the time of each of the four primings from each of the three plots. The same set of plots supplied the leaves at all four primings. Immediately after picking, the leaves were protected as carefully as possible from wilting, weighed fresh in the laboratory and their outlines traced on paper for area measurements.

The data show that treatment with phosphate alone (plot 19) produced, in each picking, a leaf surface greater than either of the other two treatments (Plots 11 and 20). Plot 19 produced the longest average stem internode of the three plots, had the highest soil ratio of phosphorus/aluminum and highest phosphorus soil test. It may have also produced the thinnest or at any rate the least dense leaves in each picking, because, as shown in Table 12, leaves from Plot 19 had the least fresh weight per unit area. It may be objected that the differences in weight per unit area in Table 12 are very small, even insignificant. Nevertheless, they show a tendency that is consistent in each picking and agree with the findings of Watson and Petrie (20).

TABLE 10. AVERAGE LEAF AREA AT PICKING TIME FOR THREE PLOTS IN RELATION TO FERTILIZATION, AVERAGE WEIGHT BASAL METER (39.37 INCHES) OF STALK AND TO PHOSPHORUS/ALUMINUM RATIO IN SOIL, FIELD A, 1941

Plot No.	Treatment—pounds per acre		Average leaf area in square inches—Leaf order of picking from base of plant				Average weight of basal meter of stalk, in ounces	Soil ratio P/Al
	48 per cent Superphosphate	Hydrated lime	1 to 3	4 to 6	7 to 10	11 to 14		
11	0	0	47	101	118	118	10.9	0.08
19	400	0	94	158	152	135	12.1	0.27
20	400	2,000	90	127	151	132	12.4	0.18

TABLE 11. AVERAGE FRESH WEIGHT OF LEAVES IN RELATION TO FERTILIZATION, STALK INTERNODE LENGTH, AND TO SOIL RATIO OF PHOSPHORUS/ALUMINUM, FIELD A, 1941

Plot No.	Treatment—pounds per acre		Average fresh weight of leaves in ounces per leaf. Leaf order of picking from base of plant				Stalk internode length, in inches	Soil ratio P/Al
	48 per cent Superphosphate	Hydrated lime	1 to 3	4 to 6	7 to 10	11 to 14		
11	0	0	0.409	0.825	0.970	0.938	2.59	0.08
19	400	0	0.808	1.210	1.076	0.885	2.75	0.27
20	400	2,000	0.772	1.019	1.090	0.892	2.72	0.18

TABLE 12. AVERAGE FRESH WEIGHT PER UNIT AREA OF LEAVES AT PICKING TIME, 1941, FOR THREE PLOTS OF FIELD A IN RELATION TO TREATMENT

Plot No.	Treatment—pounds per acre		Fresh weight per unit area of leaves, in ounces per square inch. Leaf order of picking from base of plant			
	48 per cent Superphosphate	Hydrated lime	1 to 3	4 to 6	7 to 10	11 to 14
11	0	0	0.00870	0.00818	0.00819	0.00793
19	400	0	0.00857	0.00767	0.00707	0.00658
20	400	2,000	0.00859	0.00800	0.00724	0.00674

Petrie (16), reviewing work on tobacco by Watson and Petrie (20), reports that increased phosphorus supply resulted in increased stem weight and leaf dry weight, and greater leaf area in proportion to dry weight, i.e., less leaf weight per unit area.

Chemical Analyses, 1941 Crop

Rapid Tests of Fresh Tissue

On September 9, three days after the fourth picking, six leaves were selected at random from each plot of Field A at the height of about node 15. A composite sample of 5 grams fresh tissue from the six midribs near the leaf base was comminuted for three minutes with a mixture of 50 ml Universal Soil Extracting Solution (14), 50 ml water and a pinch of decolorizing charcoal in a Waring Blendor and filtered. The filtrate was tested by Dr. M. F. Morgan according to methods described in Bulletin **450** (14) of this Station. Average concentrations for replicate plots of the nutrient constituents thus determined are presented in Table 13.

Whether differences of concentration in Table 13 are significant remains to be determined statistically. However, in the fresh tissue from plots with the highest phosphate treatments there was less nitrogen as both nitrate and ammonia, more phosphorus, less manganese and aluminum than in tissue from plots which received either no treatment or lime alone. Reduced nitrogen content of vegetative tobacco tissue, as phosphorus supply was greater at flowering time, has been observed by other workers (16 and 20). Midrib tissue from the fifteenth leaf of treatment Number 9, with the highest lime and phosphate application, showed less than half the manganese content of similar tissue from check plots. Untreated plots also produced the highest proportion of black tobacco in the first two pickings (lowest six leaves), while in the same pickings the least proportion of black tobacco came from treatment Number 9, as shown in Table 5. Whether the data of Table 13 are significantly different or not, they do show the approximate concentration range of nutrients in the fresh midrib of the fifteenth leaf and also illustrate the utility of the Universal Soil Testing System in rapid chemical analysis of plant tissue.

Analysis of Fermented Leaf and Dry Stalk

Leaf. The content of iron and manganese was ascertained[1] in leaf samples of available top grades, of the lowest grade known in the trade as KVB, and of other intermediate grades in all four pickings. Leaves were commercially sorted from plots 1 and 24, which had produced the least proportion of first prime black tobacco, and from plots 11 and 9, which produced the greatest proportion of first prime black tobacco on Field A in 1941. The manner of preparation and analysis of the samples were as described elsewhere (7). The Griffin (5) and official methods were used for the determination of iron, and the official A. O. A. C. (4) method for the determination of manganese.

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[1] Analyses by Dr. E. M. Bailey and the writer, Connecticut Agricultural Experiment Station. New Haven.

TABLE 13. RAPID CHEMICAL ANALYSIS[1] OF FRESH MIDRIB OF APPROXIMATELY FIFTEENTH SHADE TOBACCO LEAF, FIELD A, SEPTEMBER 9, 1941

No.	Treatment Pounds per acre		Parts per million of green midrib								
	48 per cent Super-phosphate	Hydrated lime	NO_3–N	NH_3–N	P	K	Ca	Mg	Al	Mn	Fe
1	0	0	1,133	150	39	7,333	4,666	866	3.3	33	T[2]
2	200	0	800	100	38	6,000	4,166	933	2.7	22	T
3	400	0	966	127	55	4,565	3,333	866	2.8	23	T
4	0	1,000	1,166	140	40	6,333	3,500	833	3.3	26	T
5	200	1,000	1,000	100	54	4,000	3,500	933	3.0	22	T
6	400	1,000	850	117	51	5,133	3,250	766	2.7	22	T
7	0	2,000	1,066	133	45	4,800	3,166	766	3.3	23	T
8	200	2,000	1,100	166	41	7,666	3,333	866	3.0	23	T
9	400	2,000	966	123	46	5,333	3,666	1,100	2.7	15	T

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[1] Analysis by Dr. M. F. Morgan, Connecticut Agricultural Experiment Station.

[2] Trace.

Analytical data for leaves are shown in Table 14 with the average tissue ratio of iron/manganese, fertilizer treatment, the average soil ratio of phosphorus/aluminum (July and September tests) for the plots concerned.

Table 14 shows with few exceptions that the darker the leaf color on any particular plot, the greater were foliar iron and manganese at any level on the stalk. For any particular leaf grade at any height on the plant, manganese content was greater on plots 9 and 11 than on plots 1 and 24. However, foliar iron content for any particular grade was not always greater on plots 9 and 11 than on plots 1 or 24. Leaves containing more than 0.16 per cent iron and 0.03 per cent manganese were black and of very inferior quality. Generally the greatest concentration of iron and manganese was found in leaves below the seventh node. Iron and manganese were generally more abundant in leaf than in stalk per unit dry weight of tissue.

In 1926 Dr. E. M. Bailey reported the inorganic analysis of one sample each of black and light tobacco. There was slightly more iron, considerably more aluminum but less manganese in the black leaves than in the light leaves.[1]

Stalk. The same 10 stalks used for measurements of internode length provided material for chemical study. Stalk tissue was analyzed from the five plots that produced the least first prime black tobacco and also from the five plots that produced the most first prime black tobacco. Basal, middle and top sections cut 95 days after setting at about the first, eighth, and fourteenth nodes, respectively, were analyzed for iron and manganese by the same technique used with leaves. Analytical data for stalks are given in Table 15.

In stalks the greatest content of iron was found in the basal section. On the contrary, manganese was more abundant in the stalk at about the fourteenth node. Manganese in all tested portions of stalks from the five plots with 64 per cent or over first prime black tobacco was consistently higher than in stalks from the five plots with 26 per cent or less first prime black tobacco. Distribution of iron in the stalk did not vary so consistently with the proportion of black tobacco in the first picking. Curiously, the iron concentration in the stalk was rather similar for all plots at about the eighth node and was notably less than the concentration at the first or fourteenth node.

Values of soil phosphorus/aluminum were generally higher and values of soil phosphorus × calcium/aluminum were much higher for the five good plots than for the five bad plots.

Iron/manganese ratio. The average ratio of iron/manganese from good plots was roughly about twice the ratio of iron/manganese in tissue from poor plots, whether leaves or stalks are considered. This suggests that plants which yielded large quantities of black tobacco had absorbed a greater amount of manganese in proportion to iron than had plants yielding more light tobacco. The average iron/manganese ratio in leaves of

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[1] Conn. Agr. Expt. Sta. Bul. **282**, p. 91, 1926.

Table 14. Iron and Manganese Content, and Iron to Manganese Ratio of Shade Tobacco Leaf in Relation to Quality Grade of Leaf, Position of Leaf on Plant, Yield of Black Tobacco, Fertilization and Phosphorus/Aluminum Ratio of the Soil Field A, 1941

Plot No.	Black tobacco per cent[1]	Treatment pounds per acre: 48 per cent Super-phosphate	Treatment pounds per acre: Hydrated lime	Ratio soil P/Al	Leaf grade	Fe, per cent dry matter, Leaf order of picking from base of plant: 1 to 3	4 to 6	7 to 10	11 to 14	Mn, per cent dry matter, Leaf order of picking from base of plant: 1 to 3	4 to 6	7 to 10	11 to 14
1	11.4	400	2,000	0.8	L	—	0.06	—	—	—	0.01	—	—
					LL	—	0.07	0.09	—	—	0.01	0.01	—
					LC	0.15	—	—	0.05	0.01	—	—	0.01
					V	0.28	0.09	0.11	0.06	0.02	0.02	0.01	0.01
					KV	0.30	—	—	—	0.03	—	—	—
					KVB	0.29	0.13	0.13	0.05	0.04	0.03	0.02	0.01
24	16.8	400	2,000	2.1	LL	—	0.07	—	—	—	0.01	—	—
					LC	—	—	0.09	0.05	—	—	0.01	0.01
					LC2	0.19	—	—	—	0.01	—	—	—
					V	0.28	0.10	0.10	0.05	0.02	0.01	0.01	0.01
					KV2	—	0.13	—	—	—	0.03	—	—
					KVB	0.36	0.22	0.10	0.05	0.04	0.03	0.02	0.01
11	87.4	0	0	0.1	LL	—	—	0.07	—	—	—	0.07	—
					LC	—	0.07	—	0.03	—	0.05	—	0.04
					YL	0.08	—	—	—	0.01	—	—	—
					YL2	0.11	—	—	—	0.01	—	—	—
					V	0.16	0.16	0.09	0.07	0.05	0.09	0.08	0.07
					KVB	0.62	0.26	0.09	0.06	0.10	0.13	0.09	0.08
9	91.3	200	2,000	0.2	L	—	—	—	0.03	—	—	—	0.02
					LC	—	0.21	0.08	—	—	0.03	0.03	—
					V	0.41	0.35	0.11	0.04	0.03	0.04	0.03	0.02
					KVB	0.46	0.33	0.12	0.06	0.06	0.06	0.04	0.07

[1] Per cent of sorted weight, first picking, leaves 1 to 3.

TABLE 14—*Concluded*

Plot No.	Black tobacco per cent[1]	Treatment pounds per acre: 48 per cent Super-phosphate	Treatment pounds per acre: Hydrated lime	Ratio soil P/Al	Leaf grade	Fe/Mn ratio in dry leaf web, Leaf order of picking from base of plant: 1 to 3	4 to 6	7 to 10	11 to 14	Average Fe/Mn ratio all pickings
1	11.4	400	2,000	0.8	L	—	6.0	—	—	7.77
					LL	—	7.0	9.0	—	
					LC	15.0	—	—	5.0	
					V	14.0	4.5	11.0	6.0	
					KV	10.0	—	—	—	
					KVB	7.2	4.3	6.5	5.0	
					Average	11.6	5.4	8.8	5.3	
24	16.8	400	2,000	2.1	LL	—	7.0	—	—	8.55
					LC	—	—	9.0	5.0	
					LC2	19.0	—	—	—	
					V	14.0	10.0	10.0	5.0	
					KV2	—	4.3	—	—	
					KVB	9.0	7.3	5.0	5.0	
					Average	14.0	7.2	8.0	5.0	8.16 Ave. good plots
11	87.4	0	0	0.1	LL	—	—	1.0	—	2.68
					LC	—	1.4	—	0.8	
					YL	8.0	—	—	—	
					YL2	11.0	—	—	—	
					V	3.2	1.8	1.1	1.0	
					KVB	6.2	2.0	1.0	0.8	
					Average	7.1	1.7	1.0	0.9	
9	91.3	200	2,000	0.2	L	—	—	—	1.5	5.58
					LC	—	7.0	2.7	—	
					V	13.7	8.8	3.7	2.0	
					KVB	7.7	5.5	3.0	0.8	
					Average	10.7	7.1	3.1	1.4	4.13 Ave. poor plots

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[1] Per cent of sorted weight, first picking, leaves 1 to 3.

TABLE 15. DISTRIBUTION OF IRON AND MANGANESE, AND IRON TO MANGANESE RATIO IN TOBACCO STALKS FROM THE FIVE BEST AND FIVE WORST PLOTS IN RELATION TO YIELD OF BLACK TOBACCO, FERTILIZATION AND SOIL TEST DATA, FIELD A, 1941

Plot No.	Black tobacco per cent[1]	Portion of stalk[2]	Mg per Kg dry stalk		Fe/Mn ratio in stalk		Treatment pounds per acre		Soil test data Average of July and Sept.		
			Fe	Mn		Average	48 per cent Superphosphate	Hydrated lime	pH	P/Al	P x Ca/Al
Best plots											
1	11.4	T	33.7	9.7	3.48	T 2.99 M 2.72 B 7.42 Ave. 4.38	400	2,000	6.25	0.8	1,558
		M	22.1	6.4	3.46						
		B	46.3	7.4	6.25						
24	16.8	T	31.2	9.9	3.15		400	2,000	6.32	2.1	3,625
		M	20.5	6.5	3.16						
		B	46.3	8.4	5.51						
13	22.5	T	25.8	7.4	3.49		400	1,000	5.61	0.7	1,181
		M	17.8	6.1	2.92						
		B	99.4	7.7	12.91						
19	24.8	T	29.1	13.0	2.24		400	0	4.98	0.3	1,164
		M	19.1	10.5	1.82						
		B	67.1	10.1	6.64						
30	25.6	T	32.4	12.6	2.57		400	2,000	6.08	0.9	1,407
		M	16.8	7.5	2.24						
		B	52.5	9.1	5.77						
Worst plots											
20	64.2	T	35.1	21.3	1.65	T 1.84 M 1.23 B 4.09 Ave. 2.39	400	2,000	5.41	0.2	296
		M	25.2	17.1	1.47						
		B	74.4	15.6	4.77						
21	76.1	T	33.8	30.0	1.13		400	0	4.80	0.1	37
		M	22.0	23.3	0.94						
		B	67.2	19.1	3.52						
33	81.0	T	32.8	13.9	2.36		0	1,000	5.43	0.3	406
		M	21.1	14.4	1.47						
		B	59.7	12.0	4.98						
11	87.4	T	73.6	48.7	1.51		0	0	4.68	0.1	32
		M	21.3	33.6	0.64						
		B	64.4	28.8	2.24						
9	91.3	T	38.9	15.3	2.54		200	2,000	5.57	0.2	369
		M	24.8	15.3	1.62						
		B	78.3	15.9	4.92						

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[1] Per cent of sorted weight, first picking, leaves 1 to 3.

[2] T, M, and B designate *top*, *mid* and *basal*, 6-cm (2.36 inches) sections of stalk cut at 94-100, 47-53, 0-6 cm, respectively, above the soil surface 95 days after setting time.

any plot was highest in the first picking and was successively less in each subsequent picking except for leaves 4 to 10 on plots 1 and 24.

The data of Table 14 do not show that any value or definite range of values of the iron/manganese ratio in tissue characterized a definite commercial leaf grade. However, the higher average ratio in plants that yielded relatively little black tobacco indicates a trend that is followed with few exceptions by the analyses of black and light tobacco reported by LeCompte (7) in Bulletin **444** of this Station. Most of the data in that study show that, in any one picking from the same field, light tobacco leaf had a higher iron/manganese ratio than dark leaf.

Somers and Shive (19) recently demonstrated the importance of balance between iron and manganese in soybean metabolism. Their plants grew well when the ratio of iron to manganese in the nutrient substrate and plant tissues fluctuated within a narrow range around 2.0, regardless of the total concentrations of these elements within the limits employed. Specific pathological symptoms developed when iron/manganese ratios either above or below this effective range prevailed in the nutrient substrate and plant tissues. The writers clearly show that iron and manganese are related in their metabolic functions. Symptoms of iron toxicity corresponded to those of manganese deficiency and symptoms of manganese toxicity corresponded to those of iron deficiency. Manganese toxicity was accompanied by a darkening of the midribs of some of the bean leaves. The darkening was somewhat purple in color.

Further study of the iron-manganese relationship in tobacco metabolism is certainly desirable. Determination is in progress of the concentrations of aluminum, copper, calcium, phosphorus and nitrogen in the leaf-grade samples from plots 1, 9, 11 and 24, Field A, 1941.

Discussion

Tobacco grown in phosphorus-deficient soils and solutions has been observed to cure a dark brown or black color by McMurtrey (11 and 12), LeCompte (7), and Morgan.[1] The direct relation of phosphorus to the formation of the black pigment in the mechanism of curing is unknown. There is no direct evidence concerning the parts played by iron and manganese among the multiple influences determining blackness in tobacco, although those metals have been found associated with black tobacco leaf in greater concentrations as the tobacco was darker, where a particular field, plot or grower was concerned (7).

Because soil phosphorus forms relatively insoluble compounds with iron, manganese and aluminum, the phosphorus supply of a soil must be so abundant as to more than saturate the demands of chemical combination with such metallic elements if plant roots are to find any phosphate available (17).

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[1] Dr. M. F. Morgan, personal communication, 1941.

The recent study by Somers and Shive (19) has shown that a balanced relationship between manganese and iron must be maintained both in plant tissues and substrate for normal growth. The tendency has been noted for the iron/manganese ratio to be lower in black tobacco than in light tobacco from a similar soil. These observations suggest that increasing the available iron in manganese-toxic soils like those studied by Morgan, Anderson and Dorsey (15) and by Jacobson and Swanback (6) might render the soil non-toxic. The possible relation of iron to manganese in the production of black tobacco cannot be disregarded.

It is entirely possible that other metals such as zinc, copper, cobalt and aluminum may be found similarly associated with black tobacco and similarly affected by phosphate and lime fertilization. *It is emphasized that this study does not identify any single factor,* for example, the lack of phosphorus or calcium or the excess of iron, manganese or other metal, *as a cause of black tobacco.* The formation of black pigment in tobacco leaf is viewed as the result of not a single cause but many causes. Livingston (8) points out that phenomena have long been regarded as arising from multiple causes. Popular appreciation of this concept is not yet extensive.

A striking fact commonly noted by tobacco workers in sorting shops is that the sorting record bears a very close relation, however obscure, to the type of land or particular farm or field where the tobacco grew. This is illustrated when one group of sorters grades all the leaves of a "light" crop from one field and later sorts the generally darker leaves of a "dark" crop from another field. Any grade from the "light" crop is often similar in name only to the grade of like name from the "dark" crop. Leaves of any grade from the dark crop, when compared with leaves of like grade name from the light crop, are actually darker to the eye and would probably be so recorded by an impartial instrument adapted to such measurements. It has been observed that when leaves of the same grade name but from two different crops, one "light," the other "dark," were mixed and sorted under comparable illumination and otherwise apparently similar conditions by a group of professional sorters, at least two different grades were established, one grade lighter than the other. All this is merely evidence to the obvious fact that commercial tobacco sorting as practiced in the Connecticut Valley does not establish absolute standards of reference for color, feel and other more illusive properties of leaf.

Had the leaves from the experimental plots been sorted in comparison to absolute standards of color and texture, the data doubtless would have shown greater contrast among treatments. Poor treatments would have appeared worse and the good, somewhat better than under the usual commercial system used in this study. A just evaluation of soil treatment as affecting leaf quality and chemical constitution would be possible if all leaves were compared with impartial absolute standards.

The lack of an impartial absolute standard of reference in commercial tobacco sorting and the evidence given in Table 5 that all dark grades in the first picking followed the same directional trend with treatments as did the black grades are facts that suggest there may be varying degrees of "black" tobacco, some not so absolutely dark as others. This is only reasonable from a physiological point of view. It is hardly to be expected that the complex biochemical reactions involved in the formation of pigment should always terminate at the same point for different plants, different soils and different curing conditions. There is serious need for standardized definition of properties of cured tobacco.

Summary

Shade tobacco from two fields was studied in 1941 and 1942 with special reference to black leaf production and soil fertilization. Both fields were new land, i.e., fallow for a long time or woodland shortly before tobacco culture, of Merrimac fine sandy and Merrimac sandy loam. Both fields were strongly acid and deficient in phosphorus and calcium when tobacco culture was begun in 1939. Black tobacco was abundant on both fields in 1939 and 1940.

Prior to the experiments, Field B received much more phosphate and lime than Field A.

Fertilization with 47 to 48 per cent superphosphate and hydrated lime evoked considerable plant response on Field A in 1941 and 1942 but only slight response on Field B in 1941.

From a commercial view, the highest crop values on Field A after a two-year period were achieved with annual application of 192 pounds per acre phosphorus pentoxide, added to the usual tobacco fertilizer. Crop value with this treatment was 46 per cent higher in the second year than the average value of untreated plots. The least proportion of black tobacco, superior leaf weight, leaf length, stalk weight, internode length, plant height and earlier flowering were observed in plants on plots treated each year with 192 pounds per acre phosphorus pentoxide either with or without lime.

Satisfactory quality was not obtained in Shade tobacco from new land until a total of about 800 pounds phosphorus pentoxide had been applied per acre.

In the second season on Field A, plots which had been limed the year before produced little black tobacco in contrast to the high proportion of black leaf from untreated plots. Plots on Field A treated with lime alone plus the usual tobacco fertilizer produced low crop value, however. Poor growth was evident in the second year on plots treated in one application in 1941 with 1,226 pounds per acre calcium oxide.

Chemical analysis of tobacco from the best and poorest plots showed that, on any particular plot, as leaf color-grade was darker, the greater were foliar iron and manganese at any level on the stalk. Manganese content of any particular grade of leaf at any height on the plant from plots which produced a large proportion of black tobacco was greater than on plots which yielded small amounts of black tobacco. Leaves containing more

than 0.16 per cent iron and 0.03 per cent manganese (dry weight basis) were black and of very inferior quality. Iron and manganese were generally more abundant in leaf than in stalk per unit weight of dry matter. Generally the greatest foliar concentration of iron and manganese was found below the seventh node. The iron and manganese content of the tobacco stalk, 95 days after setting, was studied at three points, namely, at about the first, eighth and fourteenth nodes. Stalks were richest in iron in the basal section, richest in manganese at about the fourteenth node. Iron concentration in the stalk at about the eighth node was rather similar in all samples from ten plots with widely differing fertility. There was consistently higher manganese in all tested portions of stalk from plots with 64 per cent or over first prime black tobacco than from plots with 26 per cent or less first prime black tobacco.

The average ratio of iron/manganese from good plots, all four pickings taken together, was roughly twice the iron/manganese ratio in tissue from poor plots. However, no definite value or range of values of the iron/manganese ratio appeared to characterize a definite commercial leaf grade.

Recommendations

New land that has been woodland or has lain fallow for a long time and is planned for tobacco culture should be sampled carefully and submitted to an experienced agronomist for study long before tobacco is actually set in the field. Several years of soil conditioning may be necessary before tobacco of satisfactory quality is obtainable. Judicious liming, as recommended in Bulletin **306** (15) of this Station, and heavy applications of available phosphorus have been found effective in eliminating black tobacco on such soils that are usually strongly acid, deficient in phosphorus and calcium, and which show high concentrations of "active" aluminum and manganese.

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