A BIOCHEMICAL STUDY OF SOIL ORGANIC MATTER AS RELATED TO BROWN ROOT ROT OF TOBACCO

By

Earle Dwight Matthews

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INTRODUCTION

Within the past few years it has become generally recognized that certain crops grown in rotation may have an undesirable effect upon the succeeding crop. This effect has been visited especially upon the tobacco plant, and in its most severe form when tobacco has been preceeded by a sod crop, particularly timothy. The roots of tobacco growing under these conditions often become discolored or decayed, and this condition has led to the use of the term "brown root rot." Although the disease has been under observation since 1916, the causal agency, or agencies, had never been satisfactorily determined. In 1930 (37) a certain fungus organism was consistently isolated from soils producing brown root rot. This fungus, identified as *Rhizoctonia bataticola* (Taub.) Butler, was in apparent association with the roots of diseased tobacco plants, although further study demonstrated that it was a common member of the soil flora. Preliminary to the present work, the writer investigated the nature of *Rhizoctonia bataticola*, and demonstrated that the organism can be the primary cause of brown root rot of tobacco. The present investigation was undertaken to show whether or not any correlation exists between the occurrence and severity of brown root rot and the biochemical nature of the crop residues which seem to aggravate the disease.

A REVIEW OF THE LITERATURE

The disease known as brown root rot of tobacco first appeared in the literature in an abstract of Johnson's work in 1919 (18). Johnson thought that the disease might be caused by certain strains of Fusarium that he isolated from infected plants, and with which he obtained mild symptoms by reinoculation of tobacco. In 1920 Clinton (10) described what he called a red root rot of tobacco, which, as Johnson, Slagg, and Murwin (21) point out, was without doubt identical with brown root rot. Chapman (8) in 1920 also recognized a distinctly new root disease of tobacco in the Connecticut Valley. No causal agent was mentioned as definitely associated with the disease in Connecticut by either of these men.

A description of the disease by Johnson (19) appeared in 1924. Later, Johnson, Slagg, and Murwin (21) noted a similarity between brown root rot of tobacco and the western tomato blight. They cited Heald (15) who earlier had ascribed this tomato disease to Fusarium. After microscopical study of a tomato disease which resembled brown root rot, Byars and Gilbert (7) reported a Rhizoctonia as the causal organism.

In the first detailed investigation of brown root rot (21) it was found that other plants were affected similarly, especially the tomato. The potato, eggplant, and pepper were listed as being affected, but to a lesser extent than tobacco. Among moderately susceptible plants were placed cowpeas, soybeans, garden beans, hairy vetch, the common clovers, and perhaps some of the grasses, such as oats, barley, wheat, rye, timothy, and corn, although the symptoms were questionable on these latter.

An unnamed Fusarium was isolated from diseased roots from tobacco soils of the Connecticut Valley and Maryland (21), but this organism failed to reproduce the disease by inoculation. A difficultly cultured Rhizoctonia was also obtained from the roots, and it likewise gave no positive results in inoculation experiments. This Rhizoctonia was described as being different from the "ordinary Rhizoctonia," and was considered by the workers to be the most probable parasite of the organisms isolated.

Further evidence obtained by these investigators pointed to a parasitic nature of the disease, which was summarized as follows:

"(1) Soil mixtures: Diseased soil mixed with healthy soil in proportions as low as 10 per cent of the former will result in the production of the disease, although the extent of the disease is roughly proportional to the quantity of diseased soil added.

"(2) Diseased roots: If diseased roots are washed free of soil, cut up, and mixed with healthy soil, typical infection can be obtained. Apparently the roots are proportionally more effective in this respect than diseased soil.

"(3) Steam sterilization: Steam sterilization of the soil effectively destroys the power of diseased soils to produce symptoms of the disease.

"(4) Formalin sterilization: Treatment of the soil with dilute solutions of formalin (1-50 or 1-100) destroys the ability of the soil to produce symptoms.

"(5) Temperature relations: The behavior of the disease on plants grown at different soil temperatures is apparently more closely related to biological than to chemical activity.

"(6) Symptoms of disease: The lesions produced on the roots and the response of the plant in general, together with a tendency to recover from the disease under changing environmental conditions, are more or less typical of parasitic root rots."

These workers noted two facts apparently unexplainable on a parasitic basis. One was that if the diseased soil was thoroughly air-dried in a thin layer, even in the shade, it almost completely lost its power of producing the symptoms of the disease. From this they concluded that the "common" types of plant parasites must be eliminated from consideration as causal agents. The other disturbing fact was the poor growth of tobacco and prevalence of brown root rot following various other crops in rotation. When tobacco followed timothy in the rotation, very poor crops with abundant brown root rot were obtained. A similar condition, but not so severe, occurred when tobacco followed clover and corn, and even less severe when the previous crop was onions, tomatoes, potatoes, or beans. It was noted that the injurious action upon succeeding tobacco was increased by growing the crops two or more years in succession before tobacco. It was also noted that the injurious agent, or at least its effects, apparently disappeared when the crops having harmful effects were not grown in rotation with tobacco for two or more years.

It would seem probable, then, that the nature of the crop residue had some specific affect upon the growth and condition of the succeeding tobacco crop. It should be noted, on the other hand, that when tobacco was grown in continuous culture or following fallow conditions, good yields were obtained and the plants were free of symptoms of brown root rot. Johnson and his coworkers considered that this crop relationship and the effects of air-drying upon the soil were contrary to any explanation based upon parasitism.

These investigators also observed that although the disease occurred on soils of both high and low organic content, all soils lost the greater part of their ability to produce brown root rot after the organic matter was removed by screening or floating out with water. This seemed to emphasize that the causal agent apparently existed principally in the organic matter present, which, of course, was constituted of the residues of previous crops.

Similar relationships concerning the harmful effects of other crops grown in rotation with tobacco have been conclusively established by Garner, Lunn, and Brown (13) and by Jones (22, 23, 24). In 1930 Johnson and Ogden (20) published a popular review of rotation experiments with tobacco, and Murwin, Clinton, and Anderson (32) reported field experiments in the Connecticut Valley. These papers further substantiate the harmful effects of certain crops, especially sod crops, when preceeding tobacco in the rotation.

Thomas (45) made an extensive study of brown root rot of tobacco following timothy from the standpoint of nitrate nitrogen and nitrification relationships. During the early part of the season on all soils which produced brown root rot, it was found that nitrate nitrogen was present in very low amounts, although abundant nitrates were sometimes found later in the season. It was noted that once tobacco became affected with the disease, there was little wisdom in applying nitrates, as the plants did not recover. Thomas invariably found large amounts of cellulose material in brown root rot soils, and that applications of cellulose to good soils resulted in low nitrate content and poor growth of tobacco which was always affected with brown root rot. However, these effects of cellulose applications could be overcome with heavy applications of fertilizers containing easily nitrifiable material. Moderate applications of such fertilizers had little effects.

From this it was reasoned that brown root rot of tobacco might be caused by invasion of the roots by fungi. The abundant energy material (cellulose) might so stimulate certain organisms that they would attack the tobacco roots in order to obtain nitrogen. It was pointed out that under these conditions tobacco growth would probably be inhibited by the lack of available nitrogen. The roots thus weakened might be more easily subject to attack by cellulose-decomposing fungi of the soil. An experiment was reported to substantiate this theory. Cellulose material in sterile quartz cultures did not produce brown root rot, but when a piece of infected root was added, typical symptoms developed. A piece of infected root alone failed to produce brown root rot in quartz cultures.

The preponderance of evidence from the literature seems to support the theory that brown root rot is of a parasitic nature, the parasite or parasites functioning as such only under certain conditions brought about by previous presence of certain crops. This possible parasitism was one of the points investigated preliminary to the present work.

PRELIMINARY INVESTIGATIONS

The prime object of the present investigation was to determine whether the effect of the preceeding crop upon succeeding tobacco is correlated with the chemical or biochemical nature of the crop residues. Preliminary to this, however, the disease itself, brown root rot, was more closely investigated. For many years plant pathologists had investigated the nature of the disease, but had never found a definite cause, parasitic or otherwise. However, the consistent isolation of the sterile fungus Rhizoctonia bataticola (Taub.) Butler from soils upon which tobacco was affected by brown root rot (37), led to a closer scrutiny of the organism and its potentialities as a disease-producing agency. This scrutiny consisted of studies of the association of the fungus with the roots of the tobacco plant, of physiological studies of the fungus itself, and of inoculation experiments attempting to reproduce the disease.

The Association of the Fungus with the Roots of the Tobacco Plant

Upon microscopic examination the roots of tobacco plants affected with brown root rot, it was often possible to observe fungus mycelia entering and within the root. In some rootlets there were found dark bodies resembling sclerotia. Plate I is a photomicrograph of one of the lighter lesions on a typical diseased rootlet, and shows dark sclerotium-like bodies. Portions of these rootlets were washed in sterile water and placed on Czapek's solution agar in petri dishes. A number of fungi developed, but in every case *Rhizoctonia bataticola* appeared on the plates, and could be isolated in pure culture for further identification. It was thus reasoned that the dark bodies were sclerotia of *Rhizoctonia bataticola* (Taub.) Butler, indicating that the fungus had actually invaded the roots.

As a basis for comparison, roots from apparently healthy plants grown in steamed soil were surface-sterilized and placed on Czapek's agar. In every case *Rhizoctonia bataticola* failed to appear. Plates in which no fungi whatever developed were inoculated with *Rhizoctonia bataticola*. Within a week the mycelium of the organism had invaded the rootlets, and at the end of 2 weeks sclerotia, most of them longitudinal in shape and very irregular, had formed within the rootlets as well as in the surrounding medium. Plate II is a photograph of one of these rootlets, showing the fungal invasion and sclerotial formation 2 weeks after inoculation.

Diseased rootlets were placed in petri dishes containing a little sterile water. In the tops of the dishes were placed pieces of filter paper also moistened with sterile distilled water. The dishes were incubated at 28° C. for several days, daily observations being made. Plate III, taken after 5 days shows fungal mycelium growing out upon the moist surface of the dish from the undisturbed rootlets. The appearance of the fungal growth, as shown in Plate III, was suggestive of a mycorrhizal relationship.

The Nature of the Fungus

To determine whether the nature and behavior of *Rhizoctonia bataticola* was consistent with conditions known to produce or to be associated with brown root rot, the fungus was investigated as follows: The effect of drying sclerotia and mycelial strands upon viability was determined, both in pure culture and in the soil; the relation of hydrogen-ion concentration to the growth and behavior of *Rhizoctonia bataticola* was determined; and the utilization of various carbohydrates, including cellulose, and of various forms of nitrogen was studied in the laboratory, using artificial media.

Although Rhizoctonia bataticola could be isolated readily from soil known to produce brown root rot or from sterilized soil subsequently inoculated, in no case was it isolated from diseased soil which had been air-dried in a thin layer for 4 days, even though the soil was then moistened and allowed to incubate for as long as 3 weeks. In only one case was Rhizoctonia bataticola isolated from soil which had been inoculated, dried, then moistened and incubated, and then only after 2 and 3 weeks of incubation. With this exception the results indicated that the fungus was destroyed or rendered non-viable by simple air-drying of the soil in which it was contained. Thus the nature of the fungus with respect to drying was entirely compatible with the findings of Johnson, Slagg, and Murwin (21), who observed that simple air-drying destroyed the power of the soil to produce the symptoms of brown root rot.

To test further the effect of drying upon the viability of Rhizoctonia bataticola, the following experiment was performed. The fungus was grown in sterile sand moistened with sterile Czapek's solution. Both three-day-old mycelium and six-day-old scleritia were removed from the sand and dried for various periods of time, at room conditions (temperature 26.5° C., humidity 71.0 per cent), and in a dessicator over concentrated sulphuric acid (temperature 27.0° C.), as suggested by the work of King, Loomis, and Hope (25). Ten minutes was sufficient time to destroy the viability of the three-day-old mycelium in every case. Drying at room conditions rapidly destroyed the viability of the six-day-old sclerotia; only 1 out of 13 grew on Czapek's agar after drying for 1 hour, and only 1 out of 14 after $1\frac{1}{2}$ hours. Killing of sclerotia was more rapid in the dessicator, only 1 sclerotium of 59 surviving after 30 minutes.

The drying was repeated using mycelia and sclerotia approximately 30 days old. In this experiment all drying was done in a dessicator over sulphuric acid. The older mycelia showed little if any more resistance to drying than did the three-day-old mycelia. The older sclerotia, on the other hand, were not completely destroyed until dried over 70 hours. See table 1.

In the course of general cultural and physiological studies of _Rhizoctonia bataticola_, a considerable variation in growth and characteristics of the organism coincided with variations in the hydrogen-ion concentrations of the media employed. It has been shown previously (21) that liming the soil, that is, decreasing its hydrogen-ion concentration, tended to increase the severity of brown root rot. The effect of reaction upon the organism investigated was therefore more carefully studied, using a well buffered medium, Waksman's (12). The results are tabulated in table 2, and given graphically in figure 1. It will be noted that a reaction of pH 3.8 entirely inhibited growth, and that the

Table 1

Viability of older sclerotia of <u>Rhizoctonia</u> <u>bataticola</u> dried in a dessicator.

Number of sclerotia dried	Time of drying, hours	Number of sclerotia viable	Per cent viability
45	1/4	44	98
52	1	36	69
65	3	39	65
34	8	21	62
58	24	31	53
52	36	21	40
45	51	13	29
38	66	4	11
35	72	1	3
39	78	0	0
46	84	0	0

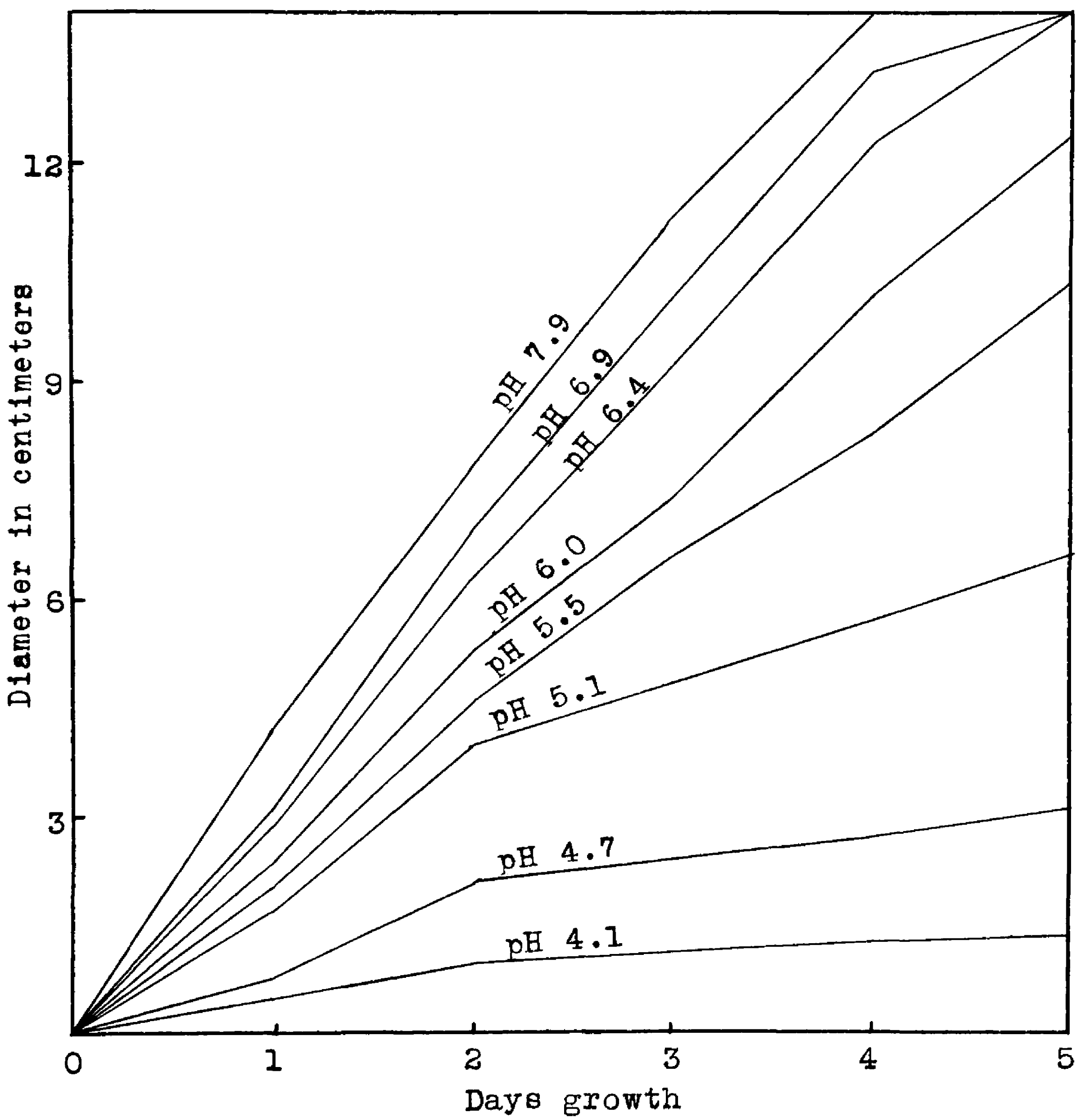
Table 2

Colony diameters of Rhizoctonia bataticola on Waksman's agar at different pH values.

pH value	Days incubation at 28° C.				
	1	2	3	4	5
	cm.	cm.	cm.	cm.	cm.
3.8	0.00	0.00	0.00	0.00	0.00
4.1	0.44	0.94	1.10	1.23	1.33
4.7	0.71	2.06	2.36	2.68	3.09
5.1	1.69	3.95	4.76	5.69	6.56
5.5	1.97	4.51	6.52	8.23	10.34
6.0	2.35	5.27	7.32	10.13	12.37
6.4	2.81	6.21	9.09	12.28	14.00
6.9	3.00	6.81	10.07	13.25	14.00
7.9	4.04	7.71	11.17	14.00	---

Figure 1

Growth curves of Rhizoctonia batatícola as measured by colony diameters on Waksman's agar at different pH values.



growth of the fungus, as measured by colony diameters, varied inversely as the hydrogen-ion concentration of the medium.

With a constant source of nitrogen (either peptone or nitrate nitrogen), and under constant conditions including hydrogen-ion concentration, *Rhizoctonia bataticola* responded almost identically to seven different sources of energy (carbohydrate). These sources were glucose, lactose, sucrose, mannite, soluble starch, starch, and cellulose. Apparently polysaccharides were as readily available to the organism as the simple sugars, and the various carbohydrates were equally efficient sources of energy.

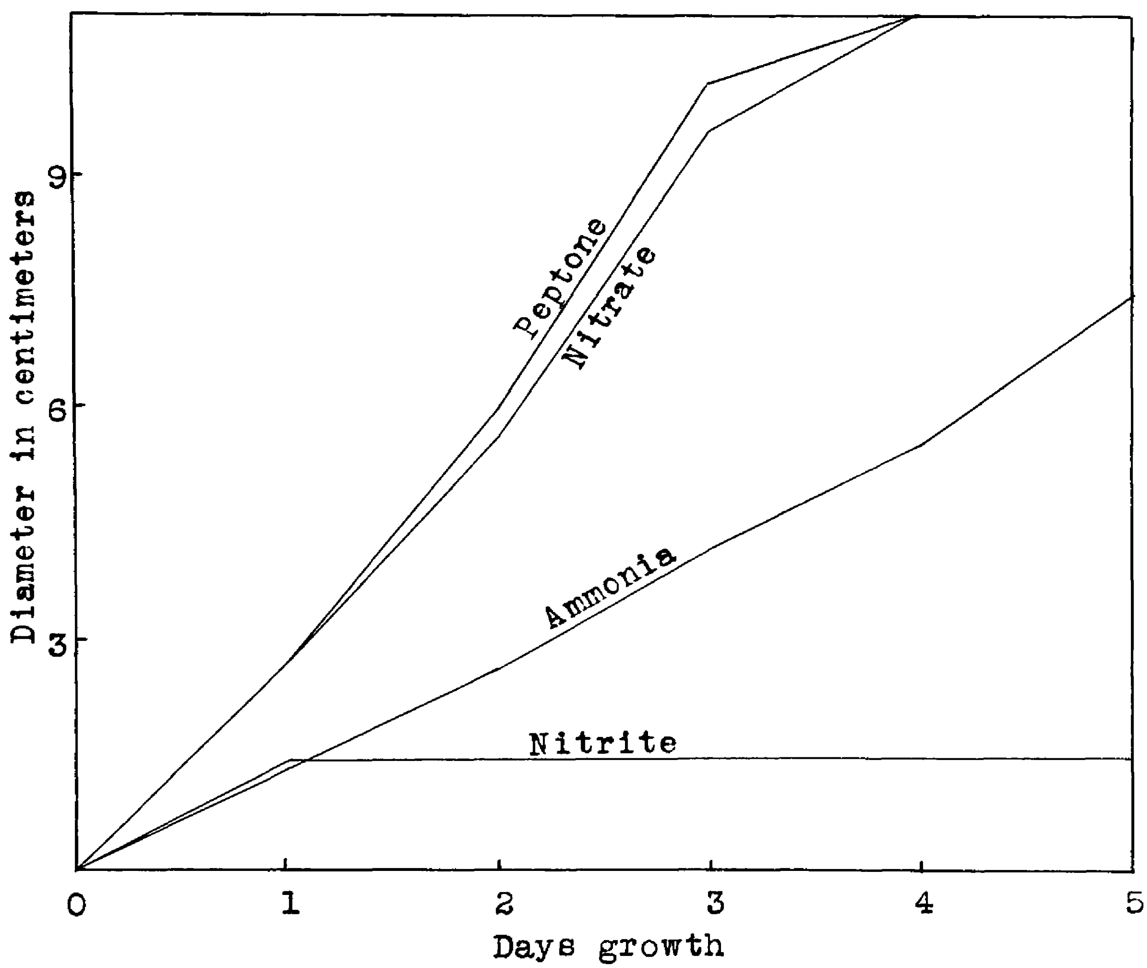
Using Norman's medium (33) with sucrose as a source of energy, the response of *Rhizoctonia bataticola* to the source of nitrogen was demonstrated. The organism responded more readily to peptone, as measured by colony diameters, than to any other source. Nitrate nitrogen gave very nearly as good results. Ammonia nitrogen gave comparatively poor results, and nitrite nitrogen negative results, as this form proved toxic to the organism after a few hours. See figure 2.

Inoculation Experiments

Since brown root rot is clearly a soil borne disease, and since *Rhizoctonia bataticola* (Taub.) Butler is a more or less common soil fungus, the soil rather than the plant was inoculated in attempts to reproduce brown root rot.

Figure 2

Growth curves of *Rhizoctonia batatícola* as measured by colony diameters on agar media with different sources of nitrogen.



A soil known to produce brown root rot was used in this experiment. Sassafras loam from a field at Cheltenham, Maryland, that had produced brown root rot of tobacco the previous season, was brought to the laboratory. Triplicate pots of this soil were prepared as follows: untreated, steam-sterilized, cellulose added, and inoculated, and various combinations of these, as shown in table 3. Cellulose was added as finely ground crude paper at the rate of 3 tons per acre. Sterilization was in the autoclave at 20 pounds pressure for 2 two-hour periods. The inocula consisted of pure cultures of Rhizoctonia bataticola (Taub.) Butler grown on slants of Czapek's solution agar. These slants were over a month old, and the contents of the tubes consisted of masses of black sclerotia. These semi-dry cultures were crushed and mixed thoroughly with the top 2 inches of the soil in the pots inoculated. The pots after treatment were transferred to the greenhouse, and a young Connecticut Broadleaf tobacco seedling set in each. No nutrients were furnished, and moisture was added as tap water, as needed. After 22 days the plants were carefully washed from the pots, and the roots floated out in water against a white background. Table 3 gives the results of examination for the presence of brown root rot on the roots of these young plants.

Brown root rot appeared on every plant that had been grown in the control pots (diseased soil). It appeared on diseased soil to which cellulose had been added, and invaria-

Table 3

The occurrence of brown root rot on tobacco in diseased soils, as influenced by steam sterilization, cellulose, and inoculation with *Rhizoctonia bataticola* (Taub.) Butler.

Soil treatment	Severity of brown root rot
Control, no treatment	Moderate
Steam-sterilized	None
Cellulose added	Moderate
Cellulose added, steam-sterilized	None
Inoculated with *R. bataticola*	Moderate
Steam-sterilized, inoculated	Moderate
Cellulose added, inoculated	Moderate to severe
Cellulose added, sterilized, inoculated	Moderate to severe

bly appeared on all plants grown in soils that had been inoculated with Rhizoctonia bataticola (Taub.) Butler. It failed to appear only when the soil had been steam-sterilized, but not subsequently inoculated. Brown root rot was most severe when additions of cellulose accompanied inoculation, even in previously sterilized soil. Thus it would seem that the disease was caused directly by Rhizoctonia bataticola, and in its most severe form in the presence of excess energy-producing material.

EXPERIMENTAL

It has been shown that Rhizoctonia bataticola (Taub.) Butler is the primary cause of brown root rot in Maryland. Investigation of the organism itself has shown not only that it probably exists endophytically in the roots of the tobacco plant, but also that the characteristics of the organism are in accordance with the conditions influencing the occurrence and severity of brown root rot. Inoculation experiments have shown that Rhizoctonia bataticola causes the disease experimentally only when the organism and cellulosic material are both present in the soil. It has also been shown that the organism causing the disease reacts differently in growth when grown artificially with different sources of nitrogen. In view of these facts and the evidence presented by existing literature, the following work was done in order to show whether or not any relationship or relationships exist between the quantitative distribution of biochemical fractions of the soil organic matter and the occurrence and severity of brown root rot. The organic fractions investigated include that soluble in benzene-alcohol, the carbohydrate portion of the organic matter, various organic nitrogenous complexes, and that portion of the organic matter containing lignin, here designated as lignin-humus. The chemical distribution of the soil nitrogen was investigated in a similar manner, the nitrogen fractions including nitrate nitrogen, other water-soluble nitrogen, ammonia nitrogen, amide nitrogen representing acid amides, nitrogen representing amino acids and various heterocyclic compounds, and the nitrogen of organic complexes resistant to prolonged acid-hydrolysis.

Arrangement and Treatment of Plots

The experimental tobacco plots from which the soils used in this investigation were taken are at the tobacco experiment station at Upper Marlboro, Maryland. The arrangement of the plots, the rotations, fertilizer practices, and yield trends are given in detail for the most part by Garner, Lunn, and Brown (13). However, for convenience, a brief description is given here. The soil is mapped as Collington. Most of it is some phase of fine sandy loam, although very small areas (only a few feet in diameter) of loamy sand are found on a few of the plots. The plots chiefly concerned in this paper are the tobacco plots of Field IV and Field V. The plot arrangement is identical in the two fields, but Field IV and Field V bear tobacco in alternate years. Thus Field IV was sampled in the year 1932 and Field V in 1933. Each field is divided into three sections.

There are twenty-one plots in each section. Then each section is divided into three sets of seven plots each. A two-year rotation of tobacco and grain with different cover crops is used on all plots, except the center plot of each subsection or set. This plot has a rotation of corn and small grain with no cover crop or fertilizer. It is called a cropping control. The grain crops of the three subsections are respectively wheat, oats, and rye. As cover crops, vetch, crimson clover, fallow (kept free of grass and weeds), fallow (corn and grain plot), cowpeas, soybeans, and grass are used in that order. See table 4. The grass cover crop is a mixture of timothy, tall meadow, oat, orchard, and Italian rye grasses. All except the grass, which is seeded with the grain crop, are planted after the grain crop and all are plowed under the following Spring before planting tobacco. All plots except the middle one of each set are fertilized with 60 pounds of phosphoric acid from precipitated dicalcic phosphate and 30 pounds of potash in the form of sulphate of potash per acre. Two-thirds of this is applied to tobacco and one-third to the grain crop. It will be noted that these

Table 4

Outline of plots and their crop rotations, with laboratory numbers and sampling dates of soils investigated.

Numbers and dates of samples								
June 1932	July 1932	Aug. 1932	June 1933	July 1933	Sept. 1933	Rotation		Cover crop
Field IV			Field V					
1	46	91	201	241	281	Tobacco,	wheat	Vetch
2	47	92	202	242	282	"	"	Clover
3	48	93	203	243	283	"	"	No crop
4	49	94	204	244	284	Corn,	"	No crop
5	50	95	205	245	285	Tobacco,	"	Cowpeas
6	51	96	206	246	286	"	"	Soybeans
7	52	97	207	247	287	"	"	Grass
8	53	98	208	248	288	"	oats	Vetch
9	54	99	209	249	289	"	"	Clover
10	55	100	210	250	290	"	"	No crop
11	56	101	211	251	291	Corn,	"	No crop
12	57	102	212	252	292	Tobacco,	"	Cowpeas
13	58	103	213	253	293	"	"	Soybeans
14	59	104	214	254	294	"	"	Grass
15	60	105	215	255	295	"	rye	Vetch
16	61	106	216	256	296	"	"	Clover
17	62	107	217	257	297	"	"	No crop
18	63	108	218	258	298	Corn,	"	No crop
19	64	109	219	259	299	Tobacco,	"	Cowpeas
20	65	110	220	260	300	"	"	Soybeans
21	66	111	221	261	301	"	"	Grass
Miscellaneous plots								
22	67	112	222	262	302	Tobacco each year		Vetch
23	68	113	223	263	303	Same, sterilized		Vetch
34	79	124	228	268	308	Tobacco every 2 years		Weeds
35	80	125	229	269	309	Tobacco every 2 years		Bare fallow
36	81	126	226	266	306	Tobacco every 3 years		Weeds
37	82	127	---	---	---	Same, sterilized		Weeds
38	83	128	227	267	307	Tobacco every 3 years		Bare fallow

plots received no nitrogen fertilizer whatever.

A few other plots were selected for sampling and subsequent analysis to compare with the plots sampled in Fields IV and V. Plot 34 has grown tobacco every other year since 1923. During the intervening years, weeds and grass were allowed to grow at random. Tobacco is grown every third year on plot 36, with volunteer weeds and grasses between tobacco crops. A complete fertilizer was used for the tobacco crop on both plots 34 and 36. Plots 35 and 38 correspond with plots 34 and 36, respectively, except that the soil was kept bare between the tobacco crops by scraping with a hoe. In 1932 portions of some of these plots were sterilized with steam. Since it is known that steam sterilization prevents brown root rot, these plots were included. They are designated as plots 23 and 37, and correspond with plots 22 and 36, respectively.

In table 4 is an outline of the plots and their rotations. The table includes the laboratory numbers and dates of the soil samples taken.

Methods of Sampling and Analysis

All plots were sampled on June 21, 1932, shortly after tobacco had been set. They were resampled at approximate one-month intervals, on July 19 and on August 23, 1932. The same or corresponding plots were also sampled in 1933, on

June 18, July 19, and September 17. Due to severe weather conditions in August of 1933, sampling was omitted at that time and the September sampling substituted.

Each sample consisted of approximately 2 kilograms of soil, composited from about twenty individual cores of soil taken with a brass tube, from the surface six inches from all parts of each plot. Each individual core was taken at an oblique angle at about ten inches from the plant and about seven inches from the center of the row. The sampling was done in this manner to prevent taking soil from the fertilizer area. All composite samples were dried at 60-65° C., passed through an 8-mesh sieve to remove any gravel and the coarser plant material, and then thoroughly mixed.

Nitrate nitrogen, water-soluble nitrogen, and ammonia nitrogen were determined on the samples thus prepared. For all other determinations, portions of the samples were ground to 40-60 mesh in a Braun pulverizer in order to obtain a more uniform distribution of the organic material in the samples. Nitrate nitrogen was determined by Harper's modification of the phenoldisulphonic acid method (14), using a 1 to 5 dilution of the soil. Ammonia nitrogen was estimated in 50-gram samples by replacement with sodium carbonate according to the aeration method of Mathews (30). For water-soluble nitrogen, 100 grams of soil were shaken at intervals for 30 minutes with 250 ml of distilled water, filtered through Whatman no. 12 paper, and two 100 ml aliquots of the filtrate taken for analysis. The Kjeldahl method modified to include nitrate nitrogen was used.

Total nitrogen and total carbon were determined on the pulverized soil. The Kjeldahl method modified to include nitrates was used for total nitrogen. Total carbon was determined by the wet-combustion, the carbon dioxide resulting being estimated by absorption in standard alkali and titration of the excess alkali with standard acid. The carbon content of the soil, multiplied by the factor 1.724, was taken as a measure of the total organic matter in the soil (48).

Further fractionation of nitrogen and the fractionation of organic matter were carried out according to a modification of the proximate system of Waksman and Stevens (48), as follows: 100-gram samples of the pulverized soil were extracted overnight with 1:1 benzene and 95 per cent ethyl alcohol in Soxhlets. The benzene-alcohol extracts were evaporated in wide-mouth 250 ml Erlenmeyer flasks and the organic carbon of the residues determined by wet combustion. Again using the factor 1.724, this carbon represents the fatty, waxy, and resinous materials in the soil organic matter. The extracted soils were carefully dried, transferred to 800 ml Kjeldahl flasks, and saturated with cold 80 per cent sulphuric acid (35 ml were found sufficient for each sample). After standing in the cold for 2 hours, 15 volumes (525 ml) of distilled water were added to each vlask, and the contents of the flasks thoroughly shaken to disintegrate any clumps of soil. The flasks were then connected to reflux condensers and hydrolysis carried out for 5 hours, after which the digests were filtered through weighed papers, and the residues washed thoroughly with distilled water,

The combined filtrates and washings were made up to 1 liter volume. Reducing sugars were determined on 200 ml aliquots. After neutralization of the aliquots with sodium hydroxide, the resultant sesquioxide precipitates were filtered off and thoroughly washed, the filtrates and washings evaporated to small volume and the large excess of sodium sulphate removed from solution by adding 3 volumes of 95 per cent ethyl alcohol, filtering, and washing with 70 per cent alcohol. Alcohol was removed by successive evaporation and addition of distilled water, and the solutions finally made up to volume. During this entire process care was taken that the solutions never became alkaline, with the aid of chlorphenol red indicator solution. Reducing sugars in the prepared solutions were determined by the official permanganate method (3), and the results were calculated and expressed as cellulose. See table 5.

Ammonia nitrogen was determined on 100 ml aliquots ofnthe same solution by distillation with magnesium oxide. The ammonia was taken as a measure of the acid amide content of the soil organic matter (48, p. 105). See table 5. Total nitrogen determinations on 100 ml aliquots included not only this ammonia (amide) nitrogen, but all of the nitrogen rendered soluble by sulphuric-acid hydrolysis. The non-amide (total hydrolyzed minus amide) nitrogen represents various amino acids and heterocyclic compounds (48, p. 109), which go to make up certain organic nitrogenous complexes.

Following the assumption of Waksman and Stevens (48), the "protein" or organic nitrogenous complexes of the soil organic matter were calculated by multiplying the percentages of nitrogen thus hydrolyzed by the factor 6.25, and dividing the result by the per cent of organic matter in the original soil, which is obtained by multiplying the percent of carbon by the equally arbitrary factor 1.724. No discussion of the assumed factors will be made here, the subject having been considered in detail elsewhere (48).

The residues from hydrolysis of the 100-gram samples of soil were carefully dried and weighed, and total carbon determined on aliquot portions. Again using the factor 1.724, the residual organic matter was calculated. Aliquots were also analyzed for total nitrogen, and the results, expressed on the basis of the original soil, multiplied by the factor 6.25, yield the residual organic nitrogenous complex. The difference between this fraction and the residual organic matter is designated as lignin-humus, since it consists of lignin and its transformation products and various other microbial complexes (48, p. 105).

Proximate Composition of Soil Organic Matter and Distribution of Soil Nitrogen

Table 5 gives the per cent of organic matter in each of the tobacco rotation plots, and the proximate composition of the organic matter in each case. The separate constituents making up the organic matter are expressed as per cent of the total. It will be seen that the total organic matter varied considerably, the minimum being 0.4624 per cent of the soil and the maximum being 1.1068 per cent of the soil, with a mean value of 0.7053 per cent for the 165 soils. The benzene-alcohol fraction varied from 2.34 per cent to 9.71 per cent of the total organic matter, with a mean of 5.49 per cent. The carbohydrate fraction of the organic matter, expressed as cellulose, varied over a similar range, the minimum, maximum, and mean values being, respectively, 3.09 per cent, 13.92 per cent, and 6.82 per cent of the total organic matter. Of the three fractions of the organic nitrogenous complexes, that calculated from the amide nitrogen varied from 4.11 per cent of the total organic matter to 13.45 per cent, with a mean value of 7.87 per cent. The organic nitrogenous complex calculated from the non-amide acid-hydrolyzed nitrogen had a minimum value of 9.67 per cent of the total organic matter, a maximum value of 22.22 per cent, and a mean of 15.44 per cent of the total organic matter. The minimum, maximum, and mean for the nitrogenous complexes resistant to sulphuric

Table 5

Per cent of total organic matter in the soils of tobacco rotation plots and its proximate percentage composition as carbonaceous, nitrogenous, and carbohydrate compounds.

				Organic nitrogenous complexes							
				Nitrogenous complexes hydrolyzed by 80% H_2SO_4 in cold (two hours) followed by boiling in 5% H_2SO_4 for five hours.							
Soil number	Total organic matter (C x 1.724)	Benzene-alcohol-soluble fraction (C x 1.724)	Carbohydrate fraction, as cellulose	Calculated from amide nitrogen (N x 6.25)	Calculated from non-amide nitrogen (N x 6.25)	Calculated from total nitrogen hydrolyzed	Calculated from nitrogen remaining insoluble after acid hydrolysis (N x 6.25)	Total organic nitrogenous complexes	Lignin-humus	Total organic matter accounted for
	per cent of soil	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent
1	.8927	4.40	8.02	6.93	11.82	18.75	10.86	29.61	42.84	84.87
2	.9968	4.47	8.41	10.82	14.43	25.25	14.09	39.34	37.96	90.18
3	.8641	6.06	5.75	7.31	11.95	19.26	13.74	33.00	40.21	85.02
4	.8216	4.26	7.92	5.63	10.99	16.62	12.72	29.34	39.28	80.80
5	.8875	6.12	7.63	6.76	15.07	21.83	15.53	37.36	38.75	89.86
6	.9154	4.97	8.15	7.37	13.93	21.30	13.99	35.29	37.80	86.21
7	.7244	5.73	8.65	8.28	15.27	23.55	14.58	38.13	37.12	89.63
8	.7244	5.00	7.62	10.44	19.59	30.03	19.06	49.09	37.73	99.44
9	.8144	6.72	7.78	9.51	16.19	25.70	18.03	43.73	41.14	99.37
10	.5544	5.86	5.84	10.60	19.95	30.55	17.47	48.02	39.40	99.12
11	.5194	6.06	6.38	10.48	17.80	28.28	16.60	44.88	41.65	98.97
12	.7344	4.45	6.69	9.53	17.44	26.97	16.00	42.97	34.56	88.65
13	.5544	5.81	6.93	7.10	21.43	28.53	19.17	47.70	38.87	99.31
14	.5496	5.59	9.20	4.54	16.26	20.80	16.60	37.40	38.48	90.67
15	.5596	5.27	6.22	8.60	17.43	26.03	18.54	44.57	42.15	98.21
16	.83.82	7.69	7.73	9.92	12.90	22.92	16.40	39.22	41.84	96.48
17	.4634	7.77	5.63	6.47	15.11	21.58	17.94	39.52	37.83	90.75

18	.4667	5.79	7.10	8.03	16.07	24.10	15.80	39.90	35.43	88.22
19	.6589	4.93	6.23	7.59	16.98	24.57	18.40	42.97	33.57	87.70
20	.6368	5.14	5.65	7.75	20.31	28.06	17.57	45.63	30.18	86.60
21	.5084	5.37	7.06	6.37	17.77	24.14	15.30	39.44	36.47	88.34
22	.6744	4.02	7.18	7.32	14.74	22.06	15.75	37.81	42.92	91.93
23	.8444	4.93	6.08	10.95	13.25	24.20	14.66	38.86	41.41	91.28
34	.7392	4.17	5.97	7.95	16.32	24.27	11.84	36.11	44.13	90.38
35	.6582	3.74	6.70	6.84	16.43	23.27	12.06	35.33	42.72	88.49
36	.7951	4.62	6.09	5.19	20.44	25.63	13.91	39.54	41.69	91.94
37	.6668	4.03	4.97	5.91	14.90	20.81	13.50	34.31	43.15	86.46
38	.5862	7.64	7.15	8.96	15.46	24.42	12.58	37.00	42.73	94.52
46	.8561	5.02	10.27	8.57	17.81	26.38	18.26	44.64	38.81	98.74
47	1.0433	6.50	9.83	11.40	12.93	24.33	16.56	40.89	36.80	94.12
48	.7555	5.13	11.08	7.03	19.43	26.46	17.93	44.39	37.38	97.98
49	.8210	6.55	10.13	7.08	18.34	25.42	14.23	39.65	36.38	92.71
50	.8919	6.26	13.92	4.62	19.21	23.83	16.26	40.09	36.21	96.48
51	.9408	7.53	11.90	4.87	16.52	21.39	15.02	36.41	36.16	92.00
52	.9400	8.20	11.29	7.60	14.39	21.99	12.82	34.81	32.41	86.71
53	.7294	5.17	11.93	10.79	20.99	31.28	18.94	50.72	30.73	99.55
54	.7770	6.98	12.95	11.50	20.27	31.77	18.98	50.75	27.44	98.12
55	.6570	6.45	10.43	9.61	18.49	28.10	18.93	47.03	35.99	99.90
56	.6491	5.41	10.55	6.89	18.50	25.39	18.20	43.59	37.37	96.92
57	.7244	5.76	11.02	7.25	22.22	29.47	18.29	47.76	36.45	100.99
58	.6946	7.52	8.92	11.79	17.32	29.11	13.97	43.08	36.20	95.72
59	.6455	6.23	12.07	11.23	16.11	27.34	13.01	40.35	36.43	95.08
60	.6886	7.18	10.02	11.34	17.49	28.83	12.56	41.39	34.17	92.76
61	.6670	6.01	9.01	9.81	17.33	27.14	13.49	40.63	38.80	94.45
62	.5994	6.64	8.83	11.27	19.70	30.97	16.16	47.13	35.08	97.68
63	.4746	6.63	9.33	9.98	14.72	24.70	13.96	38.66	38.92	93.54
64	.6296	6.77	9.26	10.82	18.86	29.69	15.98	45.67	37.44	99.14
65	.6258	6.72	9.32	9.79	16.88	26.67	14.88	41.55	37.09	94.68
66	.5734	7.17	10.17	9.16	16.46	25.62	11.88	37.50	38.84	93.68
67	.6630	4.18	4.89	9.52	16.21	25.73	16.97	42.70	44.96	96.73
68	.7451	3.91	3.99	8.39	17.70	26.09	14.51	40.60	44.77	93.27
79	.8661	6.14	4.93	6.92	10.75	17.67	12.68	30.35	46.81	88.23
80	.7127	6.87	3.41	6.75	20.08	26.83	15.52	42.35	40.40	93.03
81	.9703	6.46	4.84	8.44	14.69	23.13	15.33	38.46	45.77	95.53
82	.5541	6.26	5.98	5.64	13.42	19.06	13.20	32.26	41.97	86.47
83	.6630	7.77	5.00	10.09	13.29	23.38	10.84	34.22	47.55	94.54
91	.8330	3.93	3.26	6.83	16.06	22.89	15.31	38.20	39.76	85.15
92	1.0030	5.20	6.28	7.16	17.64	24.80	16.57	41.37	46.94	99.79
93	.9258	2.34	4.91	5.06	12.83	17.89	14.72	32.61	44.78	84.64
94	.8054	4.25	5.64	5.27	14.20	19.47	15.29	34.76	43.43	88.08
95	.8823	3.58	6.22	5.88	14.88	20.76	15.73	36.49	48.79	95.08
96	.9320	2.63	6.33	5.97	14.69	20.66	14.82	35.48	45.30	89.74
97	.8823	2.81	6.41	5.24	12.89	18.13	12.11	30.24	48.54	88.00
98	1.0740	2.47	7.19	6.81	16.35	23.16	14.22	37.38	43.06	90.10
99	1.1068	3.30	6.33	5.53	14.04	19.57	14.12	33.69	45.36	88.68
100	.8713	3.04	4.44	4.73	13.77	18.50	12.98	31.48	44.93	83.89
101	.7785	3.85	5.39	7.47	12.13	19.60	11.80	31.40	48.29	88.93

102	.8989	2.44	4.99	5.70	15.09	20.79	19.40	40.19	46.43	95.05
103	.8496	3.49	4.94	7.43	13.61	21.04	12.96	32.00	44.69	85.12
104	.7179	3.97	7.87	4.61	12.54	17.15	16.05	33.20	44.60	89.64
105	.7858	4.01	5.57	6.04	16.07	22.11	12.81	34.92	42.25	86.75
106	.8003	4.50	5.51	5.93	17.10	23.03	13.82	36.85	42.10	88.96
107	.6137	3.91	4.18	5.09	9.67	14.76	12.42	27.18	48.65	83.92
108	.5375	3.26	5.36	5.70	11.51	17.21	14.53	31.74	54.57	94.93
109	.7292	3.80	5.31	4.60	12.77	17.37	13.63	31.00	42.42	82.53
110	.7341	2.79	6.20	4.94	14.05	18.99	16.37	35.36	41.33	85.68
111	.6848	4.19	6.65	5.57	15.79	21.36	14.59	35.95	41.25	88.04
112	.6982	3.94	3.09	8.41	16.65	25.06	13.34	38.40	39.66	85.09
113	.8765	5.08	3.61	6.49	17.61	24.10	13.76	37.86	44.90	91.45
124	.8599	3.02	4.79	5.67	15.84	21.51	10.98	32.49	43.82	84.22
125	.6961	3.88	3.88	8.35	14.81	23.16	11.94	35.10	43.59	86.45
126	.9834	6.09	5.26	4.89	16.91	21.80	11.63	33.43	40.69	85.47
127	.6137	4.86	5.40	5.70	13.55	19.25	10.80	30.05	46.88	87.19
128	.6961	8.53	3.76	7.63	13.83	21.46	11.04	32.50	48.78	93.67
201	.6573	5.92	6.33	9.79	14.80	24.59	15.69	40.28	39.76	92.29
202	.6087	4.13	7.36	13.45	17.03	30.48	20.13	50.61	38.99	101.09
203	.5379	6.21	5.69	11.16	13.60	24.76	13.64	38.40	37.62	87.92
204	.6092	6.02	7.09	6.36	12.95	19.31	21.03	40.34	40.17	93.62
205	.8263	7.18	6.75	9.00	13.54	22.54	13.76	36.30	36.13	86.36
206	.8300	5.93	5.55	9.11	17.19	26.30	14.38	40.68	41.31	93.47
207	.6363	3.39	9.21	8.05	17.29	25.34	17.58	42.92	39.20	94.72
208	.7201	4.19	5.37	10.76	21.43	32.19	15.19	47.38	38.22	95.16
209	.7382	4.09	8.66	8.81	19.13	27.94	14.71	42.65	37.51	92.91
210	.5512	3.78	5.39	8.85	14.63	23.48	18.37	41.85	38.91	89.93
211	.5222	7.03	7.97	10.26	13.04	23.30	17.00	40.30	39.99	95.29
212	.8003	4.27	6.64	10.45	15.23	25.68	13.20	38.88	38.00	87.79
213	.7740	3.52	6.86	9.29	16.00	25.29	11.79	37.08	41.01	88.47
214	.7192	4.33	8.55	8.00	14.94	22.94	16.51	39.45	39.24	91.57
215	.7698	5.11	4.88	9.50	13.72	23.22	12.50	35.72	42.67	88.38
216	.74.42	3.20	4.47	7.38	14.30	21.68	14.61	36.29	43.40	87.36
217	.5232	7.08	5.52	5.26	14.57	19.83	15.89	35.72	41.99	90.31
218	.5239	4.53	6.75	8.39	12.65	21.04	16.91	37.95	41.36	90.59
219	.6479	6.04	7.66	7.24	12.73	19.97	17.65	37.62	38.75	90.07
220	.6877	4.79	7.10	6.45	12.00	18.45	16.18	34.63	40.94	87.46
221	.6120	6.14	8.14	9.49	12.50	21.99	17.46	39.45	39.02	92.75
222	.6910	9.48	5.75	6.78	16.46	23.24	13.61	36.85	36.89	88.97
223	.8299	4.30	4.19	7.76	16.68	24.44	15.48	40.22	32.39	81.10
226	.7823	5.09	6.19	7.20	18.48	25.68	14.35	40.03	37.77	89.08
227	.6806	5.88	4.56	7.90	14.47	22.37	14.10	36.47	37.83	84.74
228	.8534	5.48	5.84	6.00	16.25	22.25	12.09	34.34	37.07	82.73
229	.7423	6.41	4.68	6.82	15.04	21.86	12.46	34.32	36.39	81.80
241	.6446	6.01	7.62	6.79	15.22	22.01	17.41	39.42	41.83	94.88
242	.6387	5.29	8.36	7.52	17.71	25.23	17.77	43.00	41.40	98.35
243	.5417	5.00	8.14	6.68	13.50	20.18	16.42	36.60	45.39	95.13
244	.5905	6.07	8.28	6.98	14.60	21.68	15.61	37.29	44.13	95.67
245	.7448	6.55	7.49	11.75	16.11	27.86	15.89	43.75	47.07	104.86
246	.7484	4.22	7.16	7.52	13.12	20.64	13.51	34.15	39.11	84.64

247	.5941	6.04	7.91	7.47	14.31	21.78	14.97	36.75	48.75	99.45
248	.7125	5.64	9.12	8.33	13.51	21.84	17.51	39.35	45.05	99.16
249	.7141	5.67	8.86	9.89	14.35	24.24	15.72	39.95	45.02	99.50
250	.5336	6.26	7.78	8.13	14.68	22.81	16.78	39.59	41.32	94.95
251	.5350	4.83	6.39	6.66	13.55	20.21	17.33	37.54	54.65	103.41
252	.6924	6.07	8.71	5.14	16.07	21.21	17.92	39.13	47.36	101.27
253	.7768	5.70	6.87	7.08	12.55	19.63	14.92	34.55	42.79	89.91
254	.6558	6.80	9.74	6.67	12.01	18.68	15.76	34.44	48.63	99.31
255	.6865	4.85	7.41	7.90	14.59	22.39	14.56	36.95	46.13	95.44
256	.6846	3.54	5.62	9.31	10.77	20.08	14.78	34.86	42.99	87.01
257	.6234	7.28	4.33	4.11	10.33	14.44	13.96	28.40	53.28	93.29
258	.5486	5.19	6.07	5.70	11.62	17.32	16.59	33.91	45.62	90.79
259	.6711	4.83	8.52	4.75	13.69	18.44	15.87	34.31	39.16	86.82
260	.6313	3.55	6.27	9.70	12.18	21.88	15.80	37.68	54.32	101.82
261	.7396	6.25	8.98	5.75	13.18	18.93	13.77	32.70	48.09	96.02
262	.5539	9.71	4.87	10.38	17.11	27.49	16.30	43.79	39.93	98.29
263	.7467	5.48	4.65	8.20	17.34	25.54	16.29	41.83	44.20	96.16
266	.7155	9.24	6.16	6.64	17.87	24.51	13.53	38.04	37.77	91.21
267	.6903	6.46	4.38	6.79	15.92	22.71	14.12	36.83	45.63	93.30
268	.6762	7.57	5.59	10.72	19.78	30.50	14.72	45.22	40.12	98.50
269	.6244	8.25	5.19	9.11	15.56	24.67	15.45	40.12	39.72	92.28
281	.6170	6.46	3.73	10.43	15.30	25.73	18.23	43.96	43.42	97.57
282	.6208	6.19	5.94	10.67	15.10	25.77	18.22	43.99	46.07	102.19
283	.5596	5.02	6.07	7.04	13.62	20.66	17.31	37.97	46.18	95.24
284	.5668	5.12	6.67	8.38	11.36	19.74	17.64	37.38	45.92	95.09
285	.6999	3.57	5.44	10.27	14.11	24.38	17.15	41.53	44.68	95.22
286	.6280	5.81	5.37	9.61	14.39	24.00	14.00	38.00	45.84	95.02
287	.7042	3.21	4.83	10.29	12.16	22.45	17.44	39.89	46.04	93.97
288	.6492	5.65	3.74	8.47	16.17	24.64	20.89	45.53	46.43	101.35
289	.6320	4.94	6.98	7.51	16.71	24.22	22.65	46.87	45.51	104.30
290	.5194	5.47	5.20	10.23	12.88	23.11	19.49	42.60	47.73	101.00
291	.5020	5.88	6.99	9.84	14.07	23.91	13.32	37.23	49.18	99.28
292	.6620	5.36	9.20	6.99	16.52	23.51	18.13	41.64	45.33	101.53
293	.6229	6.97	8.44	10.03	16.96	26.99	16.26	43.25	40.91	99.57
294	.6263	5.48	6.93	6.79	16.96	23.75	15.27	39.02	47.76	99.19
295	.5920	6.35	7.57	9.29	15.73	25.02	19.43	44.45	45.22	103.59
296	.6310	6.39	6.77	7.13	15.15	22.28	14.26	36.54	48.30	98.00
297	.4624	7.35	5.15	6.49	15.82	22.31	15.68	37.99	45.72	96.21
298	.4943	6.76	7.20	7.20	15.55	22.75	19.22	41.97	45.58	101.51
299	.5820	4.88	7.13	7.73	16.82	24.55	16.32	40.87	45.26	98.14
300	.6355	6.67	9.24	8.55	14.75	23.30	17.11	40.41	43.51	99.83
301	.6018	5.27	7.21	8.83	14.23	23.06	17.55	40.61	44.48	97.57
302	.6630	7.32	4.34	5.75	16.44	22.19	11.58	33.77	47.00	92.43
303	.7620	6.54	3.97	7.87	16.68	24.55	14.14	38.69	48.19	97.39
306	.7113	7.01	4.56	7.68	14.13	21.81	11.76	33.57	51.94	97.08
307	.6744	8.20	4.48	8.16	14.72	22.88	14.32	37.20	46.03	95.91
308	.8279	6.95	4.57	6.72	16.60	23.32	11.44	34.76	52.84	99.12
309	.7179	8.66	4.51	6.27	15.52	21.79	11.30	33.09	47.40	93.66
Mean	.7053	5.49	6.82	7.89	15.44	23.31	15.33	38.64	42.12	93.07

acid hydrolysis were 10.80 per cent, 22.65 per cent, and 15.33 per cent of the organic matter, respectively. The values for the total nitrogenous complexes hydrolyzed by sulphuric acid were 14.44 per cent, 32.18 per cent, and 23.31 per cent, while those for the total of all of the organic nitrogenous complexes were 27.19 per cent, 50.75 per cent, and 38.64 per cent, respectively. The lignin-humus fraction of the soil organic matter varied from 30.18 per cent to 54.75 per cent of the total, with a mean value of 42.12 per cent.

Because of the small amounts of organic matter concerned, the assumptions made as to the organic matter and "protein" factors (48), and the multiplicity of errors of determination, the sum of the organic matter accounted for varied considerably from 100 per cent. The mean value for the 165 soils was 93.07 per cent, with a minimum recovery of 81.10 per cent and a maximum recovery of 104.86 per cent of the total organic matter. The mean figure, 93.07 per cent, is comparable to the figure of Waksman and Stevens, who obtained a mean value of 91.61 per cent recovery of the organic matter in the analysis of 11 samples by the system of proximate analysis upon which the present work is based.

Waksman and Stevens (48, p. 110) have pointed out that if the factors 1.72 and 6.25 for organic matter and "protein" are constant and correct, and that if the soil has

a constant carbon/nitrogen ratio of 10, the "protein" or organic nitrogenous complexes will always theoretically constitute 36.34 per cent of the total organic matter. Similarly calculated, if the organic matter factor is constant and correct at 1.724, the organic nitrogenous complexes would constitute 36.25 per cent of the total organic matter. In the present work, few of the soils examined have a carbon/nitrogen ratio as wide as 10, the mean value of this ratio for the 165 soils being 8.75. Consequently, the organic nitrogenous complexes would be expected to constitute a greater percentage of the organic matter. The mean value for the organic nitrogenous complexes actually determined for the 165 soils is actually 38.64 per cent. Assuming that for the number of determinations involved the errors of determination compensate for themselves, it would seem that the factors 1.724 and 6.25 are not constant and correct for the soils here investigated. One or the other may be incorrect, or perhaps both. If we further assume one factor, 1.724, to be constant and correct, using the value for the organic nitrogenous complexes observed, 38.64 per cent, and the observed value for the carbon/nitrogen ratio, 8.75, the value calculated for the "protein" factor in these soils becomes 5.83 instead of 6.25. Likewise, if the factor 6.25 is assumed correct and constant, the organic matter factor thus calculated becomes 1.849.
The actual values for these factors for the soils here under consideration probably lie somewhere between the assumed

values and the values calculated as above.

Table 6 gives the per cent of nitrogen in each of the tobacco rotation plots, and the percentage distribution of each of the fractions of nitrogen. Total nitrogen varied considerably, from a minimum of 0.0268 per cent to a maximum of 0.0853 per cent of the soil, with a mean value of 0.0480 per cent. Nitrate nitrogen showed great variation, in some cases being entirely absent. The maximum value for nitrate nitrogen was 10.96 per cent of the total nitrogen, and the mean for the 165 soils was 2.35 per cent of the total. Water-soluble nitrogen exclusive of nitrate varied from 0.00 to 10.25 per cent, with a mean of 1.42 per cent of the total nitrogen. Ammonia nitrogen was less variable, the minimum being 0.41 per cent and the maximum 8.65 per cent, with a mean of 3.23 per cent of the total nitrogen. Of the nitrogen of the organic complexes hydrolyzed by sulphuric acid, the amide nitrogen had a minimum, maximum, and mean of 10.58 per cent, 28.32 per cent, and 18.48 per cent, respectively, while the corresponding values for non-amide nitrogen were 25.32 per cent, 47.68 per cent, and 36.41 per cent, respectively, of the total nitrogen. For the total hydrolyzed nitrogen, the minimum value was 44.53 per cent of the total nitrogen, the maximum value was 67.32 per cent, and the mean was 54.89 per cent of the total nitrogen. These values for the nitrogen in complexes resistant to hydrolysis were 27.32 per cent, 51.66 per cent, and 36.15 per cent, respectively, while those

Table 6

Chemical distribution of nitrogen in the soils of tobacco rotation plots.

Soil number	Total nitrogen	Nitrate nitrogen	Water-soluble nitrogen exclusive of nitrate	Ammonia nitrogen	Nitrogen in organic complexes: Nitrogen made soluble by 80% H_2SO_4 in cold (2 hours) followed by boiling in 5% H_2SO_4 for 5 hours: Amide nitrogen	Non-amide nitrogen	Total nitrogen rendered soluble	Nitrogen remaining insoluble after sulphuric acid hydrolysis	Total nitrogen in organic complexes	Total nitrogen accounted for
	per cent of soil	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent of total	per cent
1	.0507	5.52	10.25	6.31	19.53	33.30	52.93	30.59	83.42	105.50
2	.0728	4.12	5.08	5.63	23.71	31.61	55.32	30.87	86.19	101.02
3	.0524	.95	6.30	5.53	19.28	31.52	50.80	36.36	87.06	99.84
4	.0450	.67	6.00	6.67	16.44	32.11	48.55	37.15	85.70	99.04
5	.0579	.86	5.01	4.84	16.58	36.96	53.54	38.10	91.64	102.35
6	.0579	1.21	10.02	6.22	18.65	35.23	53.88	35.40	89.28	106.73
7	.0481	.62	6.44	5.61	19.96	36.80	56.76	35.13	91.89	104.56
8	.0648	4.63	7.56	7.48	18.60	35.03	53.63	34.10	87.73	107.40
9	.0678	4.57	4.87	5.01	18.28	31.12	49.40	34.66	89.06	98.51
10	.0478	.84	6.07	6.07	19.66	37.03	56.69	32.43	89.12	102.10
11	.0417	.72	6.23	7.18	20.88	35.47	56.35	33.09	89.44	103.57
12	.0537	.93	6.15	5.21	20.86	38.17	59.03	35.01	94.04	106.33
13	.0453	1.77	6.62	6.62	13.91	41.97	55.88	37.53	93.41	108.42
14	.0361	.00	8.86	7.76	13.29	39.61	52.90	40.44	93.34	109.86
15	.0503	6.16	4.77	5.96	15.31	31.03	46.34	33.00	79.34	96.23
16	.0660	3.79	1.52	5.15	20.15	26.21	46.36	33.33	79.69	90.15
17	.0326	.31	.00	8.59	14.72	34.36	49.08	40.80	89.88	98.78

18	.0347	.29	.00	8.65	17.29	34.58	51.87	34.01	85.88	94.82
19	.0482	1.04	.42	5.60	16.60	37.14	53.74	40.25	93.99	101.05
20	.0526	1.33	.00	5.32	15.02	39.35	54.37	34.03	88.40	95.05
21	.0440	.00	1.36	6.36	14.09	39.32	53.41	33.86	87.27	94.99
22	.0482	3.73	1.04	5.60	16.39	32.99	49.38	35.27	84.65	95.02
23	.0669	5.38	.00	5.53	22.12	25.56	47.68	29.21	77.29	88.20
34	.0503	1.69	.30	4.17	18.69	38.37	57.06	27.87	84.83	91.08
35	.0441	3.06	.00	4.31	16.32	39.23	55.55	28.73	84.28	91.65
36	.0622	4.02	.00	3.21	10.62	41.80	52.42	28.42	80.84	88.07
37	.0439	.91	2.05	5.69	14.39	36.21	50.59	32.74	83.33	91.98
38	.0410	2.44	1.22	4.39	20.49	35.36	55.85	28.88	84.73	92.78
46	.0704	4.69	.28	2.13	16.67	34.66	51.33	35.52	86.85	93.95
47	.0853	9.03	.00	1.88	22.28	25.32	47.60	32.42	80.02	90.93
48	.0629	.95	.80	1.75	13.50	37.36	50.86	34.45	85.31	88.81
49	.0560	.36	.89	2.50	16.61	43.03	59.64	33.39	93.03	96.78
50	.0623	1.44	.97	4.01	10.58	43.99	54.57	37.24	91.81	98.23
51	.0559	2.50	.00	2.86	13.12	44.48	57.60	40.43	98.03	103.39
52	.0602	.83	1.66	2.66	19.11	36.18	55.29	32.23	87.57	92.67
53	.0683	5.86	3.81	3.22	18.43	35.87	54.30	32.36	86.66	99.55
54	.0741	9.58	1.08	2.70	19.29	34.01	53.30	31.85	85.15	98.51
55	.0515	.97	.00	1.75	19.61	37.74	57.35	38.64	95.99	98.71
56	.0459	.22	.65	1.52	15.60	41.85	57.45	41.18	98.63	101.02
57	.0556	1.62	.18	1.62	15.11	46.32	61.43	38.13	99.56	102.98
58	.0485	2.27	.00	1.65	27.01	39.69	66.70	32.02	98.72	102.64
59	.0430	.93	.23	1.40	26.98	38.69	65.67	31.24	96.91	99.47
60	.0493	8.92	.00	1.83	25.35	39.09	64.44	28.07	92.51	103.26
61	.0459	8.71	.00	2.40	22.80	40.30	63.10	31.37	94.47	105.58
62	.0479	.42	2.08	2.08	22.57	39.45	62.02	32.36	94.38	98.96
63	.0308	.32	3.90	3.90	24.60	36.30	60.90	34.41	95.31	103.43
64	.0475	1.05	2.31	2.31	22.94	39.99	62.93	33.89	96.82	102.49
65	.0446	1.34	2.47	2.91	21.98	37.90	59.88	33.41	93.29	100.01
66	.0368	1.36	5.98	2.17	22.83	41.04	63.87	29.62	93.49	103.00
67	.0546	9.16	.00	1.28	18.50	31.50	50.00	32.99	82.99	93.43
68	.0592	4.56	1.52	4.90	16.89	35.64	52.53	29.19	81.72	92.70
79	.0463	3.89	1.29	1.73	20.73	32.18	52.91	37.95	90.86	97.77
80	.0565	1.95	2.33	1.95	13.63	40.53	54.16	31.40	85.56	91.74
81	.0702	6.55	.00	1.71	18.66	32.46	51.12	33.86	84.98	93.24
82	.0338	.00	2.37	5.62	14.79	35.21	50.00	34.53	84.53	92.52
83	.0442	3.32	.00	1.90	25.35	33.41	58.76	27.32	86.08	91.30
91	.0570	6.14	2.98	1.58	15.96	37.55	53.51	35.79	89.30	100.00
92	.0765	9.41	2.09	1.70	15.03	37.00	52.03	34.77	86.80	100.00
93	.0519	.77	.58	1.16	14.45	36.61	51.06	42.00	93.06	95.57
94	.0474	.53	.53	1.48	14.34	38.61	52.95	41.56	94.51	97.05
95	.0490	1.22	2.45	2.04	16.94	42.86	59.80	45.31	105.11	110.82
96	.0577	1.57	1.39	1.56	15.42	37.96	53.38	38.30	91.68	96.19
97	.0480	.63	1.25	1.88	15.42	37.92	53.34	35.63	88.97	92.73
98	.0723	7.74	2.49	1.38	16.18	38.87	55.05	33.75	88.80	100.41
99	.0684	10.96	.00	2.50	14.31	36.35	50.66	36.55	87.21	100.67
100	.0455	.88	1.10	1.54	14.50	42.20	56.70	39.78	96.48	100.00
101	.0453	.44	.66	.88	20.53	33.34	53.87	32.45	86.32	88.30

102	.0540	.55	.74	.55	15.19	40.19	55.38	51.66	107.04	108.88
103	.0486	1.03	1.44	.41	20.78	38.07	58.85	30.66	89.51	92.39
104	.0367	.82	.27	1.91	14.44	39.24	53.68	50.23	103.91	106.91
105	.0527	9.30	2.09	1.33	14.42	38.33	52.75	30.55	83.30	96.02
106	.0524	8.59	.00	1.15	14.50	41.79	56.29	33.78	90.07	99.81
107	.0310	.97	.00	6.13	16.13	30.64	46.77	39.35	86.12	93.22
108	.0308	.65	.00	6.49	15.91	32.14	48.05	40.58	88.63	95.77
109	.0426	.70	.70	4.22	14.79	34.98	49.77	37.32	87.09	92.71
110	.0432	.93	2.31	2.31	13.43	38.19	51.62	44.51	96.13	101.68
111	.0416	.72	.24	1.44	14.67	41.58	57.25	38.43	95.68	98.08
112	.0515	10.87	.00	.97	18.25	36.12	54.37	28.85	83.22	95.06
113	.0636	5.03	.00	6.13	14.31	38.84	53.15	30.36	83.51	94.67
124	.0482	3.32	.00	1.20	16.23	45.22	61.45	31.29	92.74	97.26
125	.0450	.89	.22	1.42	20.58	36.67	57.25	29.49	86.74	89.27
126	.0635	2.99	.16	2.83	12.13	41.89	54.02	28.76	82.78	88.76
127	.0325	.92	.31	3.08	17.23	40.92	58.15	32.43	90.58	94.89
128	.0402	1.99	.25	2.24	21.14	38.31	59.45	30.52	89.97	94.45
201	.0486	4.53	1.02	.82	21.19	32.02	53.21	33.95	87.16	93.53
202	.0536	4.85	.93	.75	24.44	30.95	55.39	36.57	91.96	98.49
203	.0339	1.77	1.18	.59	28.32	34.54	62.86	34.59	97.45	100.99
204	.0406	1.48	.49	.25	15.27	31.08	46.35	50.49	96.84	99.06
205	.0557	3.23	.90	.72	21.36	32.14	53.50	32.67	86.17	91.02
206	.0582	3.26	.35	1.55	20.79	39.23	60.02	32.82	92.84	98.00
207	.0453	1.32	.89	1.32	18.10	38.85	56.95	39.51	96.46	99.99
208	.0551	4.54	1.37	1.45	22.50	44.82	67.32	31.76	99.08	106.44
209	.0508	6.69	.00	.98	20.48	44.48	64.96	34.21	99.17	106.84
210	.0374	1.87	.80	1.07	20.86	34.49	55.35	43.31	98.66	102.40
211	.0346	1.73	.58	.37	24.77	31.50	56.27	41.04	97.31	99.99
212	.0521	2.69	.00	.81	25.68	37.43	63.11	32.44	95.55	99.05
213	.0479	3.13	1.04	2.50	24.01	41.34	65.35	30.48	95.83	102.50
214	.0465	.86	1.51	2.58	19.79	36.98	56.67	40.86	97.53	102.58
215	.0460	4.35	.00	3.04	25.44	36.74	62.18	33.48	95.66	103.05
216	.0466	4.72	.21	2.57	18186	36.54	55.40	37.34	92.74	100.27
217	.0321	2.18	1.56	2.18	13.71	38.00	51.71	41.43	93.14	99.06
218	.0329	1.52	.30	1.52	20.97	31.61	52.58	42.25	94.83	98.17
219	.0424	2.83	.47	2.12	17.69	31.13	48.82	43.16	91.98	97.40
220	.0438	2.97	.46	2.05	16.21	30.14	46.35	40.64	86.99	92.47
221	.0440	1.14	1.59	1.82	21.13	27.82	48.95	38.86	87.81	92.36
222	.0483	2.48	2.48	4.16	15.53	37.68	53.21	31.18	84.39	93.51
223	.0601	2.83	2.66	4.27	17.19	36.94	54.13	34.95	89.08	98.84
226	.0551	1.81	1.09	4.02	16.33	41.95	58.28	32.56	90.84	97.76
227	.0467	1.71	.65	4.00	18.48	33.84	52.32	32.96	85.28	91.64
228	.0532	1.13	1.13	3.81	15.41	41.73	57.14	31.03	88.17	94.24
229	.0485	.62	.82	5.01	16.64	36.70	53.34	30.39	83.73	90.18
241	.0488	3.07	.00	3.89	14.35	32.17	46.52	36.80	83.32	90.28
242	.0516	3.29	.39	3.88	14.92	35.07	49.99	35.19	85.18	92.74
243	.0387	1.03	1.03	4.65	14.99	30.23	45.22	36.77	81.99	88.70
244	.0380	.26	1.05	4.21	17.37	36.31	53.68	38.39	92.07	97.59
245	.0573	2.09	1.05	3.48	24.44	33.51	57.95	33.05	91.00	97.62
246	.0436	2.06	1.15	4.13	20.64	36.01	56.65	37.11	93.76	101.10

247	.0340	.15	.15	4.60	20.40	39.08	59.48	40.89	100.37	107.27
248	.0530	2.64	.75	3.21	17.92	29.06	46.98	37.66	84.64	91.24
249	.0570	3.68	.17	2.63	19.83	28.77	48.60	31.51	80.11	86.59
250	.0414	.97	.72	3.62	17.39	31.41	48.80	35.51	84.31	89.62
251	.0382	.52	1.31	2.88	14.92	30.37	45.29	38.84	84.13	88.84
252	.0504	1.59	.99	3.17	11.31	35.32	46.63	39.40	86.03	91.78
253	.0448	1.79	.81	3.35	19.64	34.82	54.46	41.38	95.84	101.79
253	.0369	1.35	1.56	3.79	18.97	34.15	53.12	43.96	97.08	103.78
255	.0452	1.99	.74	3.54	19.19	35.45	54.64	35.38	90.02	96.29
256	.0407	2.46	.00	3.69	25.06	28.99	54.05	39.78	94.83	99.98
257	.0301	1.00	1.33	4.98	13.62	34.22	47.84	46,25	94.09	101.40
258	.0300	.67	1.33	4.33	16.67	34.00	50.67	48.53	99.20	105.53
259	.0391	1.53	.49	3.07	13.04	37.60	50.64	43.58	94.22	99.31
260	.0401	1.75	.50	3.49	24.44	30.67	55.11	39.80	94.91	100.65
261	.0405	.98	1.79	3.95	16.79	38.52	55.31	40.22	95.53	102.25
262	.0413	1.94	.72	1.94	22.32	36.80	59.12	35.06	94.18	98.78
263	.0526	2.28	.38	6.01	18.61	39.36	57.97	36.96	94.93	103.60
266	.0502	1.59	.40	5.50	15.21	40.93	56.14	31.00	87.14	94.63
267	.0452	1.11	1.32	1.83	16.61	38.95	55.56	34.55	90.11	94.37
268	.0520	1.73	1.34	2.11	22.31	41.16	63.47n	30.63	94.10	99.28
269	.0461	.87	1.08	2.32	19.81	33.84	53.65	33.59	87.24	91.51
281	.0457	1.09	.48	3.72	22.54	33.04	55.58	39.39	94.97	100.26
282	.0513	.97	.58	3.12	20.66	29.24	49.90	35.28	85.18	89.85
283	.0389	.51	.25	3.34	16.20	31.36	47.56	39.85	87.41	91.51
284	.0404	.50	1.00	3.73	18.91	25.62	44.53	39.80	84.33	89.56
285	.0541	1.48	.55	3.14	21.26	29.20	50.46	35.49	85.95	91.12
286	.0418	1.57	1.62	3.35	23.11	34.59	57.70	33.65	91.39	96.89
287	.0508	.00	.98	3.15	22.83	26.97	49.80	38.68	88.48	92.61
288	.0519	.58	.77	3.28	16.95	32.37	49.32	41.81	91.13	95.76
289	.0494	.81	.61	3.04	15.38	34.21	49.59	46.36	95.95	100.41
290	.0387	.26	.52	3.10	21.96	27.65	49.61	41.86	91.47	95.35
291	.0314	.32	.64	3.82	25.16	35.99	61.15	34.08	95.23	100.01
292	.0450	1.11	.22	2.22	16.45	38.89	55.34	42.67	98.01	101.56
293	.0444	1.13	.23	2.93	22.52	38.06	60.58	36.49	97.07	101.36
294	.0423	.00	1.18	3.07	16.08	40.18	56.26	36.17	92.43	96.68
295	.0445	.90	.67	2.92	19.78	33.48	53.26	41.35	94.61	99.10
296	.0369	.81	.81	2.98	19.51	41.46	60.97	39.02	99.99	104.59
297	.0268	.75	1.12	2.99	17.91	43.66	61.57	43.28	104.85	109.71
298	.0324	.31	.93	3.09	17.59	37.96	55555	46.91	102.46	106.79
299	.0392	1.02	.26	3.32	18.36	39.95	58.31	38.78	97.09	101.69
300	.0413	1.21	.00	3.39	21.06	36.32	57.38	42.13	99.51	104.11
301	.0389	.26	1.29	3.08	21.86	35.22	57.08	43.19	100.27	104.90
302	.0367	1.63	1.37	2.85	16.68	47.68	64.36	33.58	97.94	103.79
303	.0489	2.04	.21	3.63	19.68	41.72	61.40	35.36	96.76	102.64
306	.0414	1.69	1.08	3.21	20.69	38.66	59.35	32.18	91.53	97.51
307	.0414	1.45	.51	3.05	21.23	38.31	59.54	37.25	96.79	101.80
308	.0470	1.91	.52	2.43	19.03	47.01	66.04	32.40	98.44	103.30
309	.0379	1.32	.97	2.12	18.90	46.79	65.69	34.05	99.74	104.15
Mean	.0480	2.35	1.42	3.23	18.48	36.41	54.89	36.15	91.04	98.04

for the sum of the nitrogen in the organic complexes were 77.29 per cent, 107.04 per cent, and 91.04 per cent, respectively, of the total soil nitrogen.

Because no assumptions were necessary in calculating the various fractions of nitrogen, the mean of the total accounted for would be expected to be much more nearly 100 per cent than was the case with the organic matter. The mean observed value for the 165 soils was 98.03 per cent, with a minimum recovery of 86.59 perccent and a maximum of 110.82 per cent. This indicates that the deviations in each case can be attributed almost entirely to the multiplicity of errors of determination. It is further indicated that these errors so compensate for themselves that the mean of the totals approaches 100 per cent, and fails to reach 100 per cent chiefly in that the summation of the errors does not compensate completely for the multiplicity of error.

Statistical Analysis of Laboratory Results and Correlation with the Occurrence and Severity of Brown Root Rot

For statistical analysis of the effects of cropping systems, especially the effects of preceeding cover crops, on the occorrence and severity of brownnroot rot, the control or corn plots and the plots without cover crops are omitted. The miscellaneous plots not included in Fields IV and V are also omitted from statistical consideration. The severity of brown root rot was estimated by direct examination of the plants in the field, and a root rot index of arbitrarily assigned numbers established to indicate the severity of the disease, as follows:

Severity	Root rot index
trace	1
mild	2
moderate	3
heavy	4
severe	5
most severe	6

Table 7 lists the root rot index numbers assigned to each of the plots considered in the statistical analysis, and designates whether or not brown root rot was present in the miscellaneous plots investigated.

The coefficient of correlation between the severity of brown root rot and the per cent of organic matter in the soil was found to be -.368 with a standard error of ±.091, which is considered significant. However, analysis of variance showed that the per cent of organic matter in the soil does not vary with the cover crop to any significant extent. This indicates that in spite of the significant correlation between brown root rot and organic matter, it is only coincidental that the most severe brown root rot occurred on plots containing the smaller amounts of organic matter. This is

Table 7

The occurrence and severity of brown root rot of tobacco in rotation plots.

No.	Root rot index	No.	Root rot index	No.	Root rot index	No.	Root rot index	No.	Root rot index	No.	Root rot index
1	2	46	2	91	2	201	3	241	3	281	3
2	1	47	1	92	1	202	3	242	3	282	3
3	a*	48	a	93	a	203	a	243	a	283	a
4	-**	49	-	94	-	204	-	244	-	284	-
5	1	50	1	95	1	205	2	245	2	285	2
6	1	51	1	96	1	206	2	246	2	286	2
7	3	52	3	97	3	207	5	247	5	287	5
8	2	53	2	98	2	208	2	248	2	288	2
9	1	54	1	99	1	209	2	249	2	289	2
10	a	55	a	100	a	210	a	250	a	290	a
11	-	56	-	101	-	211	-	251	-	291	-
12	1	57	1	102	1	212	1	252	1	292	1
13	1	58	1	103	1	213	1	253	1	293	1
14	3	59	3	104	3	214	4	254	4	294	4
15	2	60	2	105	2	215	3	255	3	295	3
16	1	61	1	106	1	216	2	256	2	296	2
17	a	62	a	107	a	217	a	257	a	297	a
18	-	63	-	108	-	218	-	258	-	298	-
19	1	64	1	109	1	219	2	259	2	299	2
20	1	65	1	110	1	220	1	260	1	300	1
21	3	66	3	111	3	221	6	261	6	301	6
22	p***	67	p	112	p	222	p	262	p	302	p
23	a	68	a	113	a	223	a	263	a	303	a
34	a	79	a	124	a	228	a	268	a	308	a
35	a	80	a	125	a	229	a	269	a	309	a
36	a	81	a	126	a	226	a	266	a	306	a
37	a	82	a	127	a	---		---		---	
38	a	83	a	128	a	227	a	267	a	307	a

*absent **corn plots ***present

verified further by the fact that on the fallow plots, containing very low amounts of organic matter, brown root rot did not occur.

Correlation coefficients were calculated for the various fractions of the organic matter and the severity of brown root rot, and in some cases these correlation coefficients were significant. However, they were invariably negative in sign, and appeared on observation to be influenced by the total organic matter. That is, in general, any given fraction was low or high on any given plot as the total organic matter content of the same plot was low or high. By using the data for the fractions expressed as percentages of the total organic matter, this influence of the amount of organic matter was eliminated, and different correlation coefficients were found. These coefficients of correlation with the severity of brown root rot were as follows:

Benzene-alcohol-soluble fraction	+.040 ±.105
Carbohydrate fraction, as cellulose . . .	-.003 ±.105
Organic nitrogenous complexes:	
Calculated from amide nitrogen	+.026 ±.105
Calculated from non-amide nitrogen . . .	-.213 ±.101
Calculated from hydrolyzed nitrogen . .	-.151 ±.103
Calculated from non-hydrolyzed nitrogen	+.020 ±.105
Total organic nitrogenous complexes . .	-.107 ±.104
Lignin-humus	+.207 ±.101

Of the above correlation coefficients, only two, that for the portion of the organic nitrogenous complexes calculated from the non-amide hydrolyzed nitrogen, and that for the lignin-humus fraction, are in any way significant, each coefficient being barely twice its standard error.

The coefficient of correlation between the severity of brown root rot and the total nitrogen content of the soil was found to be -.386 with a standard error of ±.090, which is significant. Analysis of variance showed that the total nitrogen content of the soil varies significantly with the cover crop.

Correlations between the various fractions of nitrogen, expressed as parts per million of the whole soil, and the severity of brown root rot were all negative, and even though some appeared to be significant, they were obviously associated with variations in the total nitrogen content of the soil. The influence of the total amount of nitrogen in the soil upon these correlation coefficients was removed by redetermining the correlation coefficients using the nitrogen fractions expressed as per cent of the total nitrogen. These coefficients of correlation with the severity of brown root rot were as follows:

Nitrate nitrogen	-.273 ±.097
Water-soluble nitrogen except nitrate .	-.039 ±.105
Ammonia nitrogen	-.009 ±.105

Nitrogen in organic complexes:

Amide nitrogen	+.152 ±.103
Non-amide hydrolyzed nitrogen . . .	-.187 ±.102
Total hydrolyzed nitrogen	-.002 ±.105
Non-hydrolyzed nitrogen	+.166 ±.103
Total nitrogen in organic complexes	+.089 ±.105

It will be noted that the only significant coefficient of correlation obtained is that between the severity of brown root rot and nitrate nitrogen. Since this coefficient is negative in sign, it indicates that low amounts of nitrate nitrogen are associated with severe brown root rot. Analysis of variance showed that variation in nitrate nitrogen with the cover crop preceeding tobacco is overwhelmingly significant. However, the fallow plots on which brown root rot did not occur were quite low in nitrate nitrogen.

Similar correlations for the first sampling each season, when brown root rot was becoming established, showed no significant differences from the correlations given above. Most of the correlations became even less significant, due to the increase in standard error because of the fewer numbers of observations. A notable exception was the carbohydrate fraction of the organic matter. As noted above, the correlation coefficient between the carbohydrate and the severity of brown root rot, for the whole season, was -.003 ±.105. For the first sampling only, however, this coefficient was

+.311 ±.165 Although statistically this correlation is not quite significant, it is interesting to note that at least in a general way a high percentage of carbohydrate in the organic matter during the early season is associated with greater severity of brown root rot.

Variations in carbon/nitrogen ratio were not significant as they concern brown root rot. The coefficient of correlation between this ratio and the severity of brown root rot was -.114 ±.104.

Considering the most significant factor yet encountered, the simple coefficient of correlation between the nitrate nitrogen and the carbohydrate content of the soil was found to be +.360 ±.092. This correlation is of course based upon carbohydrate and nitrate expressed as parts of the whole soil, and not as parts of the total organic matter and total nitrogen, respectively. The whole soil is the only common basis for these two factors.

Using this correlation coefficient, the partial coefficient of correlation between the severity of brown root rot and the carbohydrate fraction, with the influence of the nitrate factor constant, was found to be -.092, which is of course insignificant. However, when the severity of brown root rot was correlated with nitrate nitrogen, with the influence of the carbohydrate factor constant, the partial coefficient of correlation became -.245, which is of course significant, although a little less so than the simple correlation coefficient between brown root rot and nitrate nitrogen (-.292 ±.096). This might indicate that although the carbohydrate fraction might be significant in that it may influence the nitrate content of the soil, it has very little if any direct bearing upon brown root rot. The multiple correlation was more significant, the coefficient being .305 ±.096, greater than either of the simple correlations involved. This shows that variations in the severity of brown root rot is associated with variations in these two soil constituents.

In considering these two constituents further, the carbohydrate/nitrate-nitrogen ratio for each of the soils investigated statistically was calculated. See table 8. In making these calculations, it was impossible to construct ratios where analyses showed nitrate nitrogen to be absent from the soil. For the expedition of this matter, the few soils (four in number) that showed no nitrate nitrogen were arbitrarily assigned one part per million of this fraction in order thatvratios other than infinity could be constructed.

The simple coefficient of correlation between the severity of brown root rot and the carbohydrate/nitrate-nitrogen ratios thus constructed was found to be +.479 ±.081. This correlation is highly significant, and indicates strongly that the more severe brown root rot is associated with wide carbohydrate/nitrate-nitrogen ratios. For the first sampling only the simple correlation coefficient was +.327 ±.163. This

Table 8

Carbohydrate/nitrate-nitrogen ratios of soils included in statistical investigation of brown root rot of tobacco.

Soil	Ratio	Soil	Ratio	Soil	Ratio
1	25.6	91	7.8	241	32.7
2	27.9	92	8.8	242	31.4
5	135.4	95	91.5	245	46.5
6	106.6	96	65.5	246	59.6
7	209.0	97	188.3	247	117.5
8	18.4	98	13.8	248	46.4
9	20.5	99	9.3	249	30.1
12	98.2	102	149.7	252	75.4
13	48.0	103	84.0	253	66.8
14	506.0	104	188.3	254	127.8
15	11.2	105	8.9	255	56.6
16	25.9	106	9.8	256	38.5
19	82.0	109	129.0	259	95.3
20	51.3	110	113.8	260	56.6
21	359.0	111	151.7	261	166.0
46	26.6	201	18.9	281	46.0
47	13.3	202	17.2	282	73.8
50	138.0	205	31.0	285	47.6
51	80.0	206	24.2	286	56.2
52	212.2	207	97.7	287	340.0
53	21.8	208	15.5	288	81.0
54	14.3	209	18.8	289	110.2
57	86.4	212	37.9	292	121.8
58	56.4	213	35.4	293	105.2
59	194.8	214	153.8	294	434.0
60	15.7	215	18.8	295	112.0
61	15.0	216	15.1	296	142.3
64	116.6	219	41.3	299	103.8
65	97.2	220	37.5	300	117.4
66	116.6	221	99.6	301	434.0

correlation is less significant than that for the whole season chiefly in that the standard error is increased because of the fewer soils involved.

To determine whether the carbohydrate or the nitrate themselves influence the correlation between the severity of brown root rot and the carbohydrate/nitrate-nitrogen ratio, partial correlation coefficients were calculated. When the severity of brown root rot is correlated with the carbohydrate/nitrate-nitrogen ratio, with the influence of the nitrate nitrogen constant, the partial correlation coefficient is +.339, as compared to the simple correlation coefficient of +.479 without the influence of the nitrate nitrogen constant. This means that if the influence of the nitrate nitrogen were actually constant, the severity of brown root rot would still vary directly with the carbohydrate/nitrate-nitrogen ratio, although not so significantly. When the severity of brown root rot is correlated with the carbohydrate/nitrate-nitrogen ratio, with the influence of the carbohydrate fraction constant, the partial correlation coefficient is +.492. This means that if the influence of the carbohydrate content of the soil were constant, the severity of brown root rot would be even more highly correlated with the carbohydrate/nitrate-nitrogen ratio. This in turn indicates that of the two factors involved, nitrate nitrogen plays by far the most important role in the severity of brown root rot, and that the carbohydrate content is of secondary importance.

The mean value of the carbohydrate/nitrate-nitrogen ratio for all soils on which brown root rot occurred was 90.95, while for the soils on which grass had been the preceeding crop, and upon which brown root rot was most severe, the mean ratio was 227.57. The mean carbohydrate/nitrate-nitrogen ratio of fallow plots, upon which there was no brown root rot, was 126.18, which is greater than the mean value for the affected plots.

DISCUSSION

The work preliminary to the chemical and statistical investigation definitely confirms Thomas' hypothesis (45) that brown root rot may be caused by an actual invasion of the roots by a fungus or fungi. *Rhizoctonia bataticola* (Taub.) Butler was demonstrated to be capable of producing the disease under conditions similar to those under which brown root rot is most severe both in the field and in pot cultures. Because the parasitism of *Rhizoctonia bataticola* seems to be facultative and dependent upon certain soil conditions, it becomes apparent why the cause of the disease had not been found previously. Johnson, Slagg, and Murwin (21) found a number of cellulose-decomposing fungi in association with the roots of tobacco affected by brown root rot, and clearly pointed out the conditions under which brown root rot was known to occur, but they were unable to show parasitism of the organisms which they isolated. Nevertheless, their work supports both the findings of Thomas (45) and of the present investigation.

Although *Rhizoctonia bataticola* is only now demonstrated to be the causal agency of brown root rot of tobacco, it has been described as the cause of root disease of tobacco by workers in various parts of the world. Reichert (36) reported that *Rhizoctonia bataticola* appeared in tobacco areas in Palestine in 1923, producing a root disease and causing a great loss of the crop. Small (39, 41) also has reported root diseases of tobacco caused by the same fungus. Uppal (47) has stated that *Rhizoctonia bataticola* is suspected of being parasitic on tobacco in Gujarat, India. Ciferri (9) reported a root disease of tobacco in Santo Domingo, at least similar to brown root rot, caused by a Rhizoctonia which he did not identify. It is problematical whether his organism was *Rhizoctonia bataticola*. In a bulletin issued by the Department of Agriculture of Ceylon in 1928 (1), *Rhizoctonia bataticola* was given as the cause of root diseases of a great number of plants, including a root rot of tobacco.

Rhizoctonia bataticola (Taub.) Butler has been amply demonstrated to be the cause of diseases similar to brown root rot on the roots of a great number of plants other than tobacco. The more prominent of these plants include cotton (1, 4, 5, 11, 26, 31, 42, 47), bean (1, 6, 27, 28, 34, 36, 38, 39), tomato (1, 29, 36, 39), eggplant (1, 29, 40), pepper (1, 29, 36), sweet potato (2, 29, 44), tea (1, 43, 46), and various species and varieties of citrus (1, 16, 35, 41).

It was pointed out that the negative correlation between organic matter and the severity of brown root rot seems to be only coincidental. This seems to be verified by the fact that although brown root rot varied significantly with the preceeding cover crop, the total organic content of the soil did not, and therefore should not vary significantly with brown root rot. The fallow plots, in which the organic content was very low, did not produce brown root rot. In the preliminary work, brown root rot was most severe when cellulose was added, increasing the organic content of the soil. These points are definite evidence that small amounts of organic matter do not increase the severity of brown root rot, and that the greater severity of brown root rot in the field is only coincidental with the low organic matter content of the soil.

It has been noted that only two fractions of the organic matter, as determined by the system of proximate analysis employed, were correlated at all significantly with the severity of brown root rot. These were the lignin-humus fraction and that part of the organic nitrogenous complexes calculated from non-amide hydrolyzed nitrogen. If highly significant, which they are not, these correlations would indicate that large percentages of non-amide complexes accompany slight severity of brown root rot, and that large percentages of lignin-humus in the organic matter accompany great severity of brown root rot. These correlations are too small to justify anything more than the barest generalizations, even though statistically they show significance.

Although a significant coefficient of correlation (-.386 ±.090) was found between the total nitrogen content of the soil and the severity of the disease, and although analysis of variance showed that total nitrogen varies significantly with the previous cover crop, there are facts which minimize the importance of the total nitrogen content of the soil. First, the plots upon which no cover crops were grown (which were omitted from statistical analysis), and which produced no brown root rot, contained even less total nitrogen than the plots on which brown root rot was most severe. Second, it has been pointed out that the correlation of brown root rot with total organic matter was probably coincidental, and since total nitrogen is highly dependent upon and correlated with total organic matter, it follows that the variations in the per cent of total nitrogen in the soil are also probably coincidental with the variations in the severity of the disease. Third, these factors were so positively correlated with each other that partial correlations with one, holding the influence of the other constant, were entirely insignificant. Fourth, the multiple correlation between the severity of brown root rot and organic matter and nitrogen was smaller than either of the simple correlations involved. This multiple correlation coefficient was .358 ±.092, as compared to -.368 ±.091 for brown root rot correlated with total organic matter, and -.386 ±.090 for brown root rot correlated with total nitrogen. This evidence seems to indicate that neither the total organic matter content nor the total nitrogen content of the soil are highly significant as they affect the severity of brown root rot of tobacco.

Of the different nitrogen fractions investigated, it must be emphasized that only the nitrate nitrogen, as per cent of the total nitrogen, was found to be significant, and that analysis of variance showed that nitrate nitrogen is highly significant in its variations with the kind of preceeding cover crop. The fact that nitrate nitrogen was low on fallow plots, which produced no brown root rot, seems to indicate, however, that large amounts of nitrate nitrogen are not necessary in preventing the occurrence of the disease, and that some other factor is probably involved. This other factor appears to be the carbohydrate fraction of the soil organic matter. As pointed out above (p. 46), the carbohydrate content of the organic matter was almost significant when only the first early-season samplings were considered. Also, the multiple correlation coefficient of .305 ±.096 between brown root rot and nitrate and carbohydrate, which is greater than the simple correlations involved, shows that the carbohydrate has some significance. The effect of the carbohydrate on the correlation between brown root rot and the nitrate content of the soil was shown in the partial correlations calculated (p. 46). It was shown that there is still a significant correlation (-.245) between brown root rot and nitrate nitrogen when the influence of the carbohydrate fraction is eliminated as a single factor. Thus the carbohydrate factor does affect the nitrate nitrogen, but probably does not affect brown root rot in a direct manner.

The importance of nitrate nitrogen as it influences brown root rot is emphasized by the correlations between brown root rot and the carbohydrate/nitrate-nitrogen ratio. Although the simple correlation coefficient was very significant, being -.479 .081, partial correlation between brown root rot and the ratio, with the influence of the constituent parts, carbohydrate and nitrate, of the ratio held constant, showed that the nitrate nitrogen rather than the carbohydrate is the most important factor in influencing the correlation between brown root rot and the carbohydrate/nitrate-nitrogen ratio.

No definite minimum value for nitrate nitrogen, below which brown root rot should occur, can be designated. However, it would seem that the critical nitrate value, if any, would be dependent in some way upon the carbohydrate/ nitrate-nitrogen ratio of the soil. To test this supposition, the partial correlation coefficient between the severity of brown root rot and nitrate nitrogen was determined. The influence of the carbohydrate/nitrate-nitrogen ratio upon that correlation was held constant. The value was found to be but -.044, as compared to -.292 for the simple correlation coefficient between brown root rot and nitrate nitrogen, as parts per million of the whole soil. This is definite evidence that the influence of variations in nitrate nitrogen upon the severity of brown root rot is vary largely dependent upon the influence of variations in the carbohydrate/nitrate-nitrogen ratio of the soil.

The results of this work seem to confirm the results of Thomas (45) in his earlier work on nitrate nitrogen and nitrification in relation to growth of tobacco and to brown root rot. However, the work of Thomas apparently indicated that carbohydrate material had a more important direct bearing upon the occurrence and severity of the disease. The present statistical analysis clearly shows that it is highly significant only in its ratio to nitrate nitrogen. It is shown further that this ratio is of significance chiefly in its influence upon the correlation between brown root rot and the nitrate nitrogen content of the soil. Nitrate nitrogen is the only single soil constituent found to be highly significant in its direct influence upon the severity of brown root rot of tobacco.

Brown root rot occurred in but one of the miscellaneous plots investigated, that planted to tobacco each year but with a hairy vetch cover crop. Only mild symptoms of brown root rot were present. Examination of the chemical data for this plot showed that only a moderate amount of carbohydrate material was present in the organic matter, and that nitrate nitrogen was quite high. The carbohydrate/nitrate-nitrogen ratio had a mean value of 12.7. Conclusions from the statistical analysis indicate that brown root rot should not occur to any extent on this plot, and as pointed out, the symptoms of the disease were quite mild.

It has been known quite generally that volunteer growth of weeds between tobacco crops does not have undesirable effects insofar as brown root rot is concerned. Plots 34 and 36 were planted to tobacco every 2 and 3 years, respectively, with volunteer weeds allowed to grow between crops. Plots 35 and 38 correspond to plots 34 and 36, but no weeds or other vegetation were allowed to grow between the tobacco crops. The data show that there were no significant differences between those plots grown to weeds and those kept in fallow condition, except that all organic and nitrogenous constituents were generally present in greater amount in the weed plots. On the average, the weed plots contained 437.4 parts per million carbohydrate material, 21.15 parts per million nitrate nitrogen, and the mean carbohydrate/nitrate-nitrogen ratio was 20.1. These values for the fallow plots were 323.0, 7.67, and 42.1, respectively. Although the fallow plots had the wider ratio, this ratio was still quite narrow as compared to those for the plots previously considered on which brown root rot was quite severe. These ratios were essentially the same when only the early samplings were considered. From the standpoint of organic matter and nitrogen analyses, there is nothing to indicate that brown root rot should occur on any of these plots.

The effect of steam sterilization was chiefly to increase all forms of organic matter and nitrogen. There was little effect on the carbohydrate/nitrate-nitrogen ratio, the mean ratio for sterilized plots being 22.3 as compared to 14.0 for the corresponding unsterilized plots. There was no lack of nitrate nitrogen in either the sterilized or unsterilized corresponding plots. Chemical analyses of organic matter and nitrogen do not reveal why sterilization prevents brown root rot. Prevention is of course probably due to the destruction of the causal organism *Rhizoctonia bataticola*.

SUMMARY

A study was made of the soil conditions or factors which might produce the disease known as brown root rot of tobacco. In preliminary investigations the common soil fungus, *Rhizoctonia bataticola* (Taub.) Butler, was shown to be capable of causing the disease. The nature of the fungus

was found to be consistent with unusual factors concerning the disease which were pointed out in the literature. These factors were the effects of drying the soil and of the residues of previous crops.

One hundred and sixty-five soils from tobacco rotation plots at Upper Marlboro, Maryland, were investigated. The amount of organic matter in each of these soils was determined, as well as the following constituents of the organic matter: the benzene-alcohol-soluble fraction, carbohydrate material, organic nitrogenous complexes as calculated from amide, non-amide acid-hydrolyzed, and hydrolysis-resistant nitrogen, and lignin-humus. The total soil nitrogen and its chemical distribution was determined, the fractions investigated being nitrate nitrogen, water-soluble nitrogen other than nitrate, ammonia nitrogen, amide nitrogen, non-amide acid-hydrolyzed nitrogen, and resistant or non-hydrolyzed nitrogen. The severity of brown root rot on ninety of these soils was estimated by direct examination of the tobacco roots, and a root rot index established. The laboratory results were analyzed statistically in conjunction with the root rot indices, in order to determine what constituents of the soil organic matter or what fractions of soil nitrogen might be correlated with the severity of brown root rot.

Nitrate nitrogen was the only single soil constituent found to be very significant in its influence upon the severity of brown root rot. The coefficient of correlation between nitrate nitrogen, as parts per million of the soil, and the severity of the disease was found to be -.292 ±.096. When nitrate nitrogen was expressed as per cent of the total nitrogen, this coefficient was -.273 ±.097. The carbohydrate fraction alone was of no significance, but the coefficient of correlation between the severity of brown root rot and the carbohydrate/nitrate-nitrogen ratio was the most significant encountered, being -.479 ±.081. Partial correlations indicated that the influence of variations in nitrate nitrogen upon the severity of brown root rot is very largely dependent upon the influence of variations in the carbohydrate/nitrate-nitrogen ratio.

It is concluded that brown root rot of tobacco in Maryland is caused by *Rhizoctonia bataticola* (Taub.) Butler. The severity of the disease is negatively correlated with the nitrate nitrogen content and the carbohydrate/nitrate-nitrogen ratio of the soil. The preceeding crop affects the succeeding tobacco crop indirectly by means of the carbohydrate/nitrate-nitrogen ratio, and directly by means of the nitrate nitrogen content of the soil.

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PLATE I

Sclerotia of *Rhizoctonia bataticola* (Taub.) Butler, within and upon a rootlet of tobacco plant affected with brown root rot.

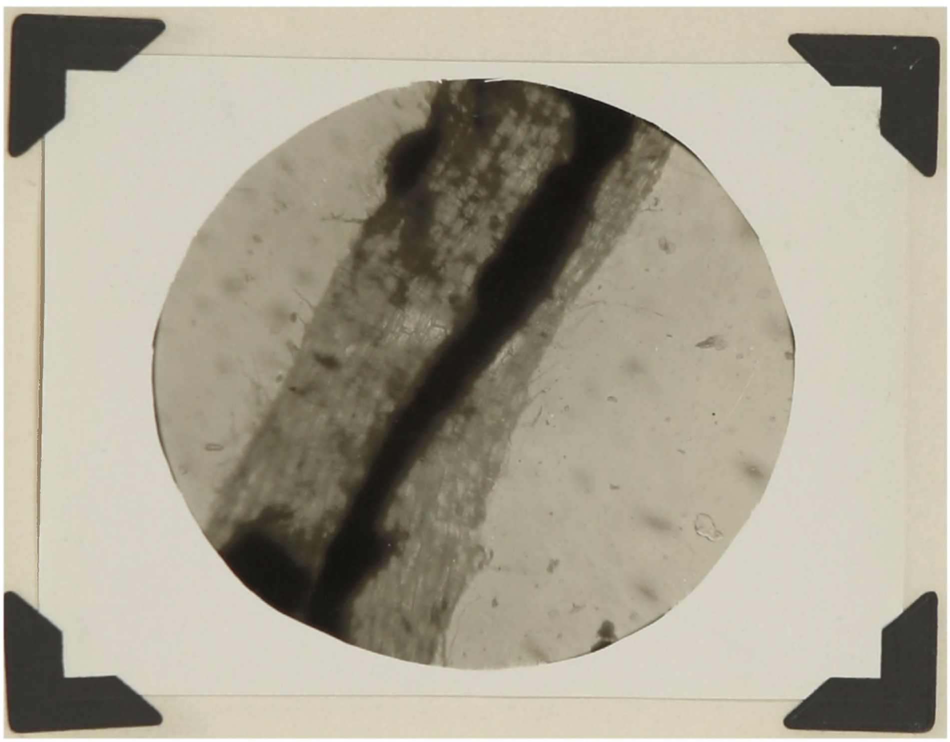

PLATE II

Invasion of tobacco rootlet by *Rhizoctonia bataticola* (Taub.) Butler in pure culture; sclerotia and mycelia within rootlet and in medium.

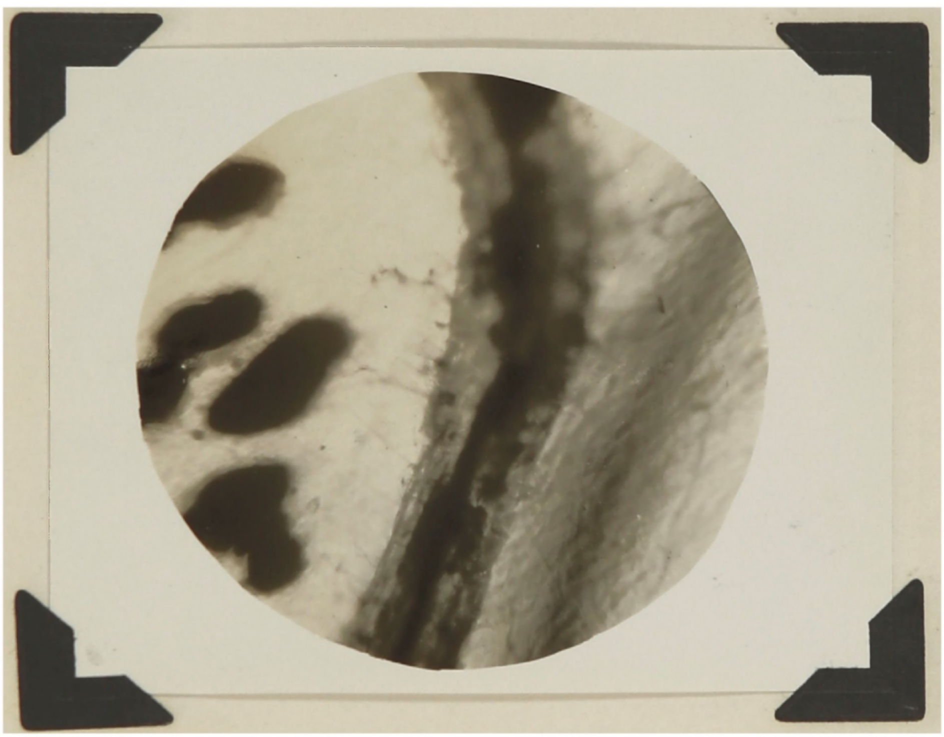

PLATE III

Tobacco rootlet affected with brown root rot, showing fungal growth upon incubation in moist chamber.