UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 842

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER September 7, 1920

THE NEMATODE DISEASE OF WHEAT CAUSED BY TYLENCHUS TRITICI

By

L. P. BYARS, formerly Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, in cooperation with the Office of Cereal Investigations

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OCCURRENCE OF THE DISEASE.

A disease of wheat caused by the nematode *Tylenchus tritici* (Steinbuch) Bastian has been known for many years in certain European countries, where it causes considerable damage. The disease manifests itself most strikingly in the wheat heads. Here rather small dark-colored galls of the same general shape as wheat kernels are formed in place of normal grains. It is also known to affect other aerial parts of the wheat plant, but does not attack the roots. In thrashing, many of the nematode galls thrash out with the wheat. Examinations by the writer of numerous samples of wheat collected during the winter of 1917–18 by the Office of Grain Standardization, of the Bureau of Markets, at mills and other places, revealed the presence of many of these galls and showed for the first time that the disease occurs to a serious extent in certain localities in the United States, particularly in Virginia. Subsequent observations in the field have shown that the pest in some instances destroys more than 40 per cent of the growing wheat. It therefore seems de-

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sirable and timely to bring to general attention the data at hand, so that the disease may be more readily recognized, its further importation and distribution prevented, and its control understood.

HISTORY.

Needham (24),[1] in 1743, in making microscopic examinations of supposedly smutted grains of wheat, found that they contained numerous motionless, eelworm larvæ instead of spores. When placed in water these larvæ soon began eellike movements; hence, there is this definite evidence of the occurrence of the disease in England at this early date. At that time Needham briefly recorded his observations and again in 1745 (25) described the results of further investigations, but was unaware of their full significance until Roffredi in 1775 (30) and 1776 (31) published the result of accurate investigations covering several years, which clearly showed for the first time the causal relation of the nematodes to the malady and shed considerable light on the life history of the parasite. In 1799, Steinbuch (34) dealt with a disease of wild grass caused by a nematode which he called *Vibrio agrostidis* and considered different from the wheat eelworm. He appears to have been the first investigator who proposed a name for the latter organism. He first refers to the parasite in these words, "und welche *Vibrio tritici* genannt werden konnte," and subsequently uses the name *Vibrio tritici* many times and in such manner as to show conclusively that it is the wheat nematode of which he is speaking. Bauer (3), in 1823, after considerable study of and experimentation with the organism, also named it *Vibrio tritici*, apparently unaware of the fact that it had been previously thus designated by Steinbuch. Although Steinbuch was the first to apply the binomial, Bauer seems to have been the first to use the name systematically for the parasite. This doubtless accounts for the fact that many later investigators cite him (Bauer) as authority for the species. Dujardin (13), in 1845, transferred the species to the genus Rhabditis. Rhabditis is a genus described by Dujardin and contains mostly free-living forms which have little in common with Tylenchus. Diesing (12), an eminent systematic helminthologist, in 1850 also made a similar classification of the parasite. He placed it in the genus Anguillula described by Hemprich and Ehrenberg. This genus contains the vinegar eel and other free-living forms. Assuming that the parasite occurred on other grasses than wheat, he accordingly called it *Anguillula graminearum* Diesing. In his classical monograph, Bastian (2) in 1864, correctly transferred the old species name as used by Steinbuch, i. e., *tritici*, to the genus Tylenchus.

[1] The numbers in parentheses refer to "Literature cited" at the end of this bulletin.

The correct designation, therefore, becomes *Tylenchus tritici* (Steinbuch) Bastian. Two years later, in 1866, Schneider (32) gives the scientific name of the organism as *Anguillula scandens*.

In view of the above brief résumé of the work of those investigators who took some part in naming the parasite, its proper synonymy chronologically recorded would seem to be as follows:

Tylenchus tritici (Steinbuch) Bastian, 1864.
  Vibrio tritici Steinbuch, 1799.
  Vibrio tritici Bauer, 1823.
  Rhabditis tritici Dujardin, 1845.
  Anguillula graminearum Diesing, 1850.
  Tylenchus tritici Bastian, 1864.
  Anguillula scandens Schneider, 1866.

Following Roffredi's (31) publication of 1776, many papers on the disease appeared in European literature, some of which have been mentioned. They were for the most part discussions as to the correctness of the observations of Roffredi, and contributed little to what was already known about the trouble until the monograph of Davaine (11) appeared in 1857. In it he gives practically all that is known to-day about the etiology of the disease, lucidly describes and illustrates the different stages in the development of the parasite, and records the results of physiological researches on the nematode which furnish a basis for its control. His work largely silenced the discussion that had prevailed in the literature as to the cause of the disease, some investigators having confused the trouble with stinking smut of wheat (*Tilletia tritici*).

Many papers appear in European literature after Davaine's publication, but in general they deal mainly with the occurrence and distribution of the disease and add little to what had previously been recorded. Marcinowski's (22) valuable contribution of 1909, however, added considerably to our knowledge of the pathogenicity and physiology of the parasite. Since that time no publications of major importance relating to the disease have been found.

In the United States the occurrence of the eelworm disease of wheat seems to have been first recognized by Johnson (19), who in 1909 recorded that it had been found in California by members of the staff of the Office of Cereal Investigations of the Bureau of Plant Industry, and had been authentically reported to him to occur in New York, Georgia, and West Virginia. There appear to have been no other records of the trouble in North America until Fromme (14) in 1917 published a short note stating that he had found it at one point in Virginia. Since that time the writer (7, 8) has reported its wide prevalence in Virginia and its limited occurrence in other States, has described the general nature of the disease, and has pointed out control measures. Later, Coleman and Regan (10) contributed to our knowledge of the disease so far as it affects the marketing and milling of wheat. A similar disease of several other grasses, thought to be due to a closely related or identical species of nematode, was reported by Bessey (4) in 1905 and later by the writer, but recent investigations indicate that the organisms producing the malady in wheat and other grasses differ physiologically, if not morphologically. The writer's acquaintance with the disease on wheat began in 1915, when minor inoculation experiments were conducted in the greenhouse with material received from China. Interest was renewed during the winter of 1917–18 as the result of finding, in cooperation with the Office of Grain Standardization, an abundance of material in the United States. Consequently, previous experiments in the greenhouse were repeated and extended, laboratory investigations carried out, and field observations made. These studies are being continued.

DISTRIBUTION.

The nematode disease of wheat is of almost world-wide distribution, having been reported from all continents except Africa. Sorouer (33), in 1913, stated that it had been found in France, Germany, Austria, Hungary, Switzerland, Italy, Sweden, Holland, England, and North America. Dr. N. A. Cobb [1] has found it many times in Australia and the writer (6) in 1917 reported its occurrence in China. Through the courtesy of Dr. C. E. Leighty, of the Office of Cereal Investigations, Bureau of Plant Industry, an examination of all samples of wheat imported by the United States Department of Agriculture for the past 25 years or more was made and, besides finding the disease in material obtained from some of the countries listed above, it was also found in importations originating in Russia, Turkestan, and India, from which places no reference to its occurrence has been seen. Averna-Sacca (1) in 1912 stated that it occurs in Brazil. From the foregoing account it is evident that the disease can exist under very different climatic and soil conditions and that it is at present of extremely wide occurrence.

Indications are that the disease has been introduced into this country. That it may be endemic in Europe is suggested by the fact that it was not reported elsewhere for nearly a century after its discovery there. It seems likely that it was first introduced into this country with importations of seed wheat from England or continental Europe. The exact native habitat of the disease, however, is an interesting and open question which may never be definitely an-

[1] In conversation with the writer, Dr. Cobb stated that he had found the trouble on wheat in Australia in 1892.

swered. In this connection it is of interest to note that a careful examination by the writer of a considerable collection of native emmer, or so-called "wild wheat" (*Triticum dicoccoides*), from Palestine failed to reveal the disease.

Within the United States the disease was first found in 1909 at Modesto, Calif., and Old Fields, W. Va. During the same year it was reported from New York and Georgia. As a result of cordial cooperation by the Plant Disease Survey of the Bureau of Plant Industry and the Office of Grain Standardization of the United States Department of Agriculture and by pathologists and other agricultural agents of various States, the writer has recently examined specimens

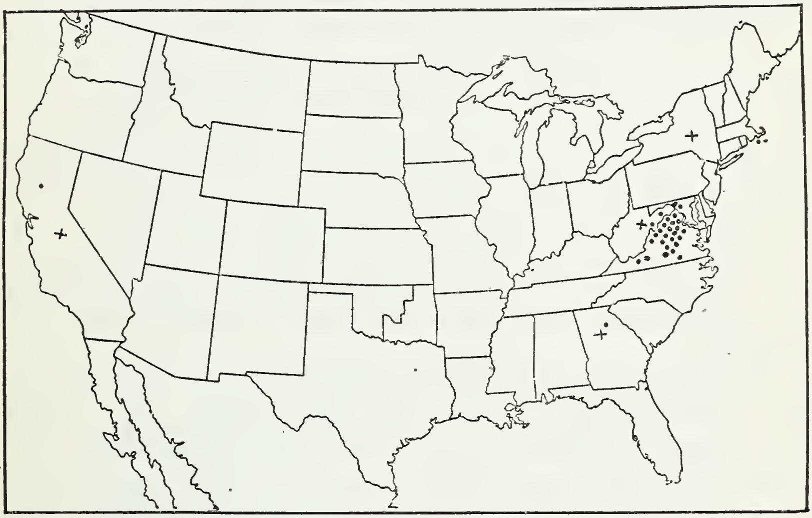


Fig. 1.—Outline map of the United States, showing the distribution of the wheat nematode. The dots represent the States where it was found during 1918, while the crosses indicate the localities in which the disease was reported in 1909.

from Red Bluff, Calif., from two counties each in West Virginia and Maryland, from one county in Georgia, and from a large number of widely separated places in Virginia. Distribution of the trouble in this country is graphically shown in figure 1. Whether the malady occurs only on the east and west coasts and not in the great wheat-growing States of the Middle West is not now known. It may be possible that the trouble exists in the Central States to a limited extent and has been merely overlooked or mistaken for stinking smut or other troubles, or it may not occur there as yet. As the disease apparently is not endemic in the United States, nor especially widespread as yet, every effort should be made not only to prevent its further importation into and spread within this country, but it should be eradicated as far as possible from localities already infested.

ECONOMIC IMPORTANCE.

Economically this wheat disease is of considerable importance in certain countries and of very minor significance in others. In central Europe, especially in Germany, where the trouble was found soon after its discovery in England, reports during the last decade indicate that the disease caused little damage. Earlier writings, however, show that it was responsible for severe losses. Haberlandt (15) in 1887, for example, reported that of 3 samples of wheat collected from as many Provinces in eastern Germany, 20 contained considerable quantities of the diseased kernels.

Doubtless improved methods of cultivation and sanitation and the general agricultural practices in combination with a knowledge of means of controlling the disease are responsible for its apparently minor economic status in central Europe in recent years. In England, where conditions seem to have been rather favorable for its development, the malady now appears to be well controlled, although, as in Continental Europe, writers during the eighteenth century report its serious prevalence at times. Judging from the examination of small lots of wheat shipped from Russia, Turkestan, and India, it occurs in these countries to a considerable extent. Reisner [1] in 1915, without knowing its cause, reported that the malady at that time was the cause of severe losses in certain parts of China.

During the past year the disease has been found to be the cause of heavy losses at certain places in the United States. Lots of wheat sent in from or collected by the United States Department of Agriculture in Virginia contained, according to count, from a fraction of 1 per cent to more than 50 per cent of the nematode galls. These findings, together with the fact that many of the galls are lost before and during thrashing and that there are many direct losses caused to the crop in the field by the disease not shown by the thrashed grain, suggest much more severe damage than that indicated by the percentage counts referred to above. During the summer of 1918 the writer found several fields in one locality in Virginia in which about half of the wheat heads were severely infected, and, in addition, many of the plants were killed.

DESCRIPTION OF THE DISEASE.

The nematode disease of wheat manifests itself only on the aerial parts of the host. It affects directly both the young and old leaves as well as the embryonic fruit, and may indirectly cause a bending or crooking of the stem. On seedlings mildly infected it usually

[1] Dr. H. J. Reisner, of the University of Nanking, Nanking, China, in a letter transmitting specimens to the Office of Cereal Investigations, states that the disease causes great damage in some Chinese Provinces.

produces a wrinkling, twisting, and rolling of the younger leaf blades, as shown in Plate I, *A* and *B*. Small raised, rounded areas may appear on the upper surface of such leaves, which lose their normal green color, become yellow, wilt, and die. Very young leaves sometimes, however, show conspicuously none of these symptoms. Occasionally they contain light-colored swellings or galls, one or more of which may be located along the midrib, on the leaf edge, or between the two, and are misshapen by an unequal lateral development. A conspicuous gall on the edge of a leaf is shown in Plate II, *A* and *B*, and apical swellings near the midrib of a young leaf 16 days after artificial inoculation are depicted in Plate II, *C*. By tearing apart these leaf galls several weeks after they have been induced, the eelworms which cause them can be found in all stages of development.

When seedlings become more severely attacked than already indicated, more pronounced evidence of abnormality appears. The young leaves become so strongly infected within the older leaf sheaths that instead of growing straight up normally they may be forced through the latter, carrying along with them the young stem. In this way stems sometimes are bent and induced to grow in an almost horizontal direction. The leaves become so wrinkled, twisted, and rolled as to lose all semblance of their natural shape. Their normal green color then disappears and finally, after wilting, the entire plant dies. Diseased leaves sometimes roll so tightly as to hold firmly the tip of the younger leaves. This results later in wrinkling and more or less telescoping of these leaves as they grow from below.

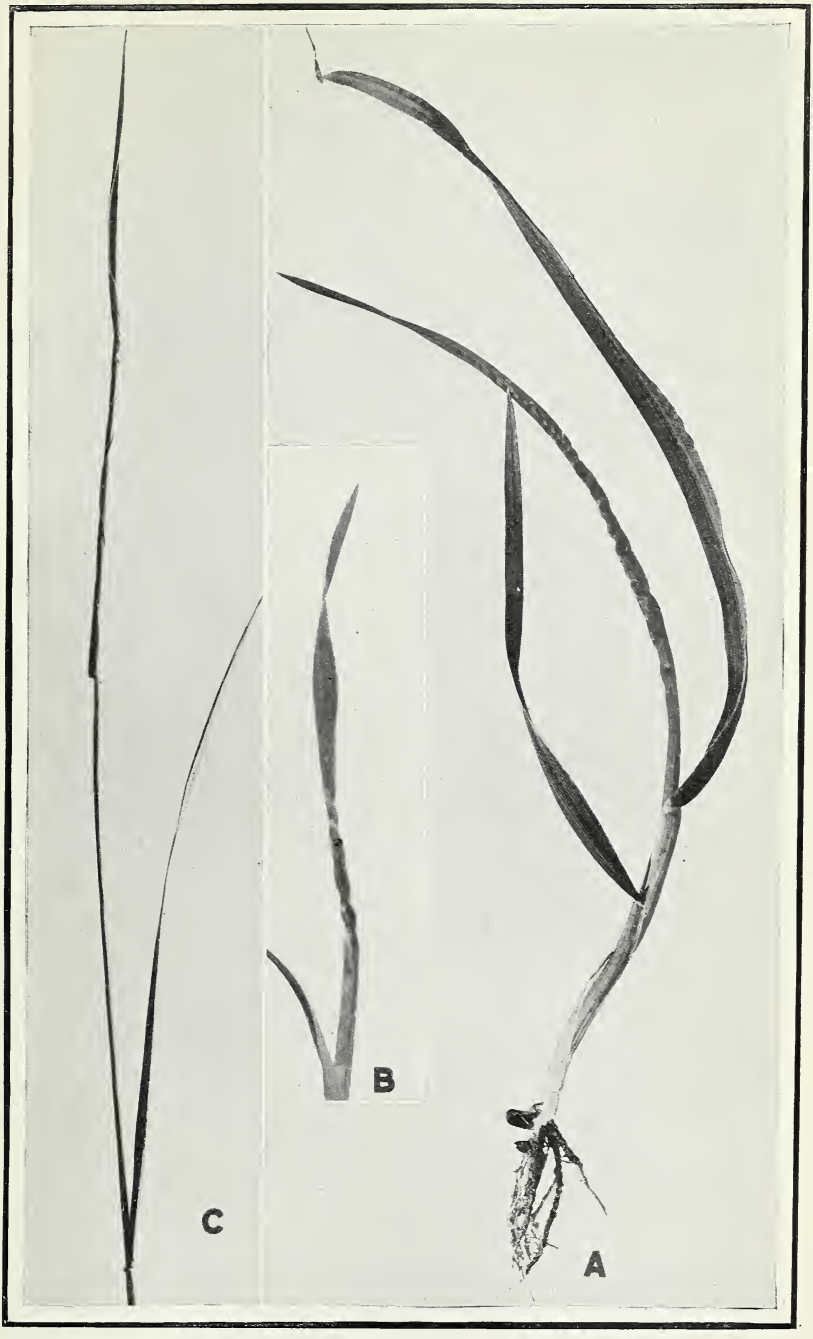
Leaf and stem symptoms of the disease occur more commonly on the seedlings than on the older plants, which leads to the general belief that the former outgrow the trouble. Marcinowski (22), however, does not agree with this, and after conducting an experiment in which 90 per cent of the infected seedlings died while still young, concluded that as a rule plants succumb before maturity when diseased at an early stage of growth. At any rate, leaf and stem symptoms of the disease are less common, as well as less noticeable, on the older plants. Maturing plants may be decidedly dwarfed, however, somewhat yellow in color, and show a curling of the upper leaves, as may be seen in Plate I, *C*.

Leaf rolling, however, which is of more frequent occurrence than the dwarfing and yellowing, is not always an indication of the nematode disease, since it may be caused by other factors. While leaf symptoms usually occur, they may be entirely absent, and yet, at the maturity of the plant, the head may be found to be severely infected.

Most positive and clear evidence of the disease can be detected by a careful examination of the heads of the plant at maturity. Diseased spikes (Pl. III) are usually reduced in size, especially in length, remain green longer and therefore mature somewhat later than the normal ones, and the glumes protrude decidedly outward and give a somewhat thickened appearance to the shortened spike. Depending upon the severity of attack, some or practically all flowering glumes on infected heads contain, in the place of normal kernels, hard, light-brown to dark-colored galls which are filled with nematodes. These galls, though usually slightly furrowed on one side, somewhat as are wheat kernels, are shorter and not uncommonly thicker. This thickness of the galls often results in the spreading of the inclosing glumes so as to expose the galls to almost full view. As a consequence of this, wheat heads thus infected may be readily detected in the field. When young, the galls are light to dark green in color. They gradually become dark brown later, as the normal wheat heads ripen. Because of a general similarity, they were in France first confused with and mistaken for "smutted" wheat and called "blé niellé," but only a simple test is necessary to distinguish the two. A smutted grain is easily crushed by a little pressure and becomes a mass of smutty powder, the black spores, whereas the galls are hard and firm and break with difficulty. In Germany the galls were first associated with the seed of cockle (*Agrostemma githago*), a weed found commonly growing in wheat. This resulted in the disease being designated there as "Radekrankheit." Only a cursory examination, however, is necessary to distinguish the smooth nematode galls from the black cockle seeds, which are covered with rows of short spines. In England the trouble is perhaps most commonly called "purples," on account of the color of the galls. Farmers and millmen in sections of this country where the disease occurs call wheat containing these galls various names, such as smutted, bunted, cockle, bin-burnt, and immature wheat. There have been instances, some of them recorded, where pathological investigators in this as well as other countries, without making microscopic examinations, wrongly identified the galls as stinking smut of wheat (*Tilletia tritici* or *T. levis*). Some of the differences in size, shape, color, and general appearance between the nematode galls and the material for which they have been mistaken are shown in Plate IV.

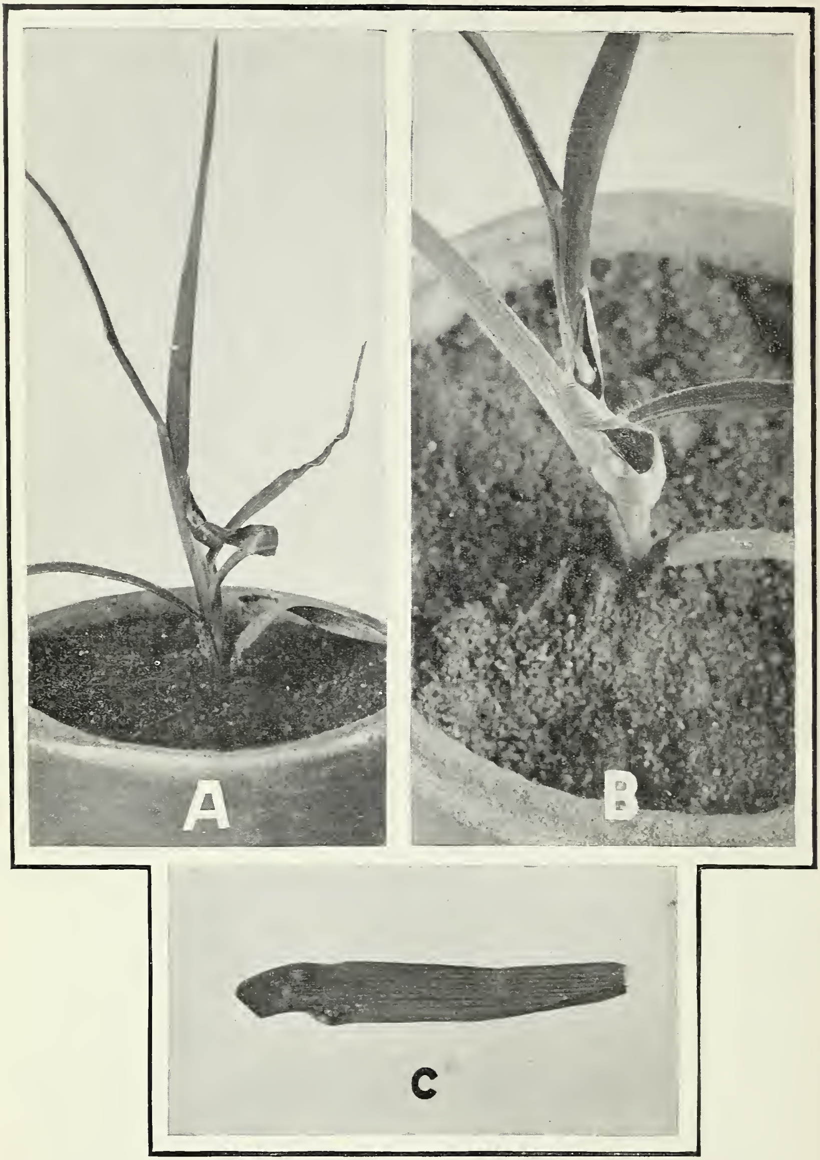
DIFFERENCES BETWEEN THIS DISEASE AND TULIP-ROOT.

It seems desirable to point out differences between the malady discussed in this paper and the so-called stem disease, or "tulip-root," of wheat and other cereals, which, while occurring in European countries, has not been reported on wheat in America. The two troubles have been confused both popularly and scientifically, doubtless owing to their occurrence together at times and



LEAVES OF WHEAT PLANTS AFFECTED BY TYLENCHUS TRITICI.

A, Month-old seedling from the greenhouse, inoculated by placing a few drops of water containing larvæ on the seed when planted. Note the abnormal wrinkling of the central leaf. *B*, Leaf from an infected greenhouse seedling, showing the characteristic wrinkling, rolling, and twisting caused by the nematodes. *C*, Part of an almost mature field-grown plant, the head of which contained many nematode galls. The upper leaf often is found tightly rolled on such plants.



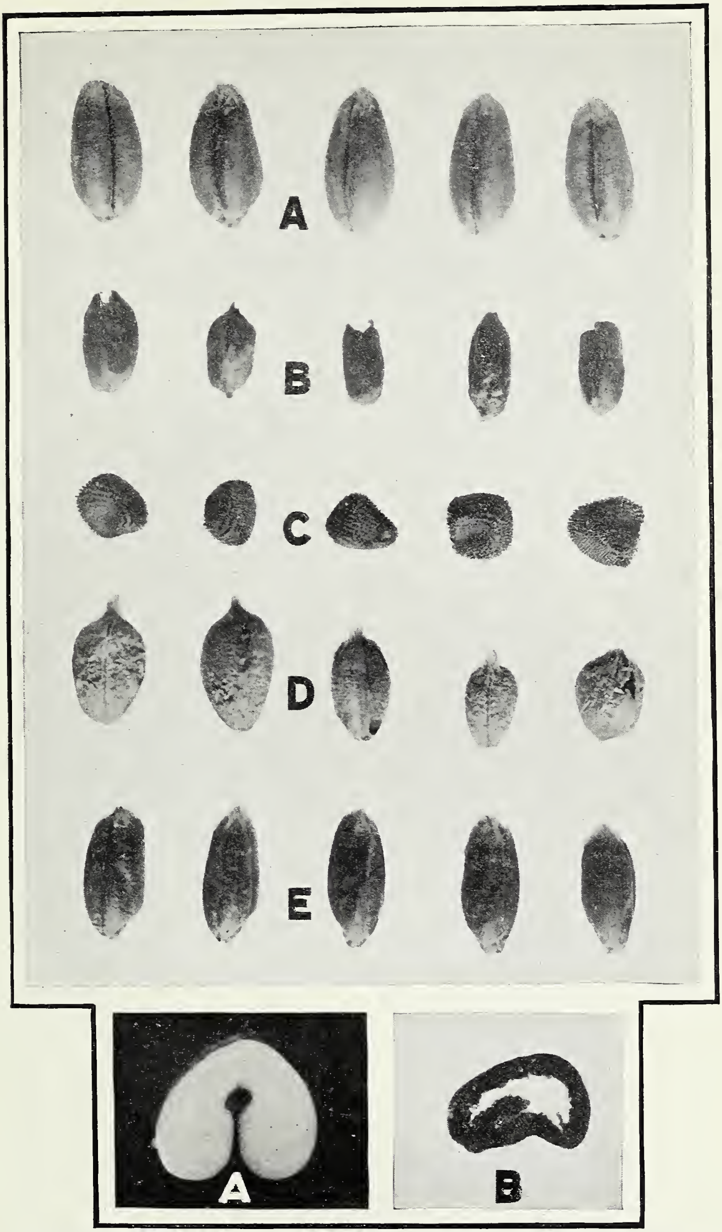
WHEAT SEEDLING AND LEAF INFECTED BY NEMATODES.

A, A five-weeks'-old wheat seedling from a greenhouse, artificially infected with *Tylenchus tritici* and decidedly dwarfed as a result. Observe that the twisted central leaf is held in the next older leaf sheathed by a nematode gall on its edge and that the small upper leaf on the right is distorted, wrinkled, and twisted. Photographed May 31, 1918. Natural size. *B*, Part of the plant pictured in *A*, enlarged to show the location, shape, and size of the nematode gall on the central leaf and contiguous portions of the infected leaf. × 2. *C*, Leaf blade of a wheat seedling, showing the one-sided apical development and small nematode galls as a result of artificial inoculation in the greenhouse. Photographed in October, 1917. × 2.

A. HOEN & CO. BALTIMORE.

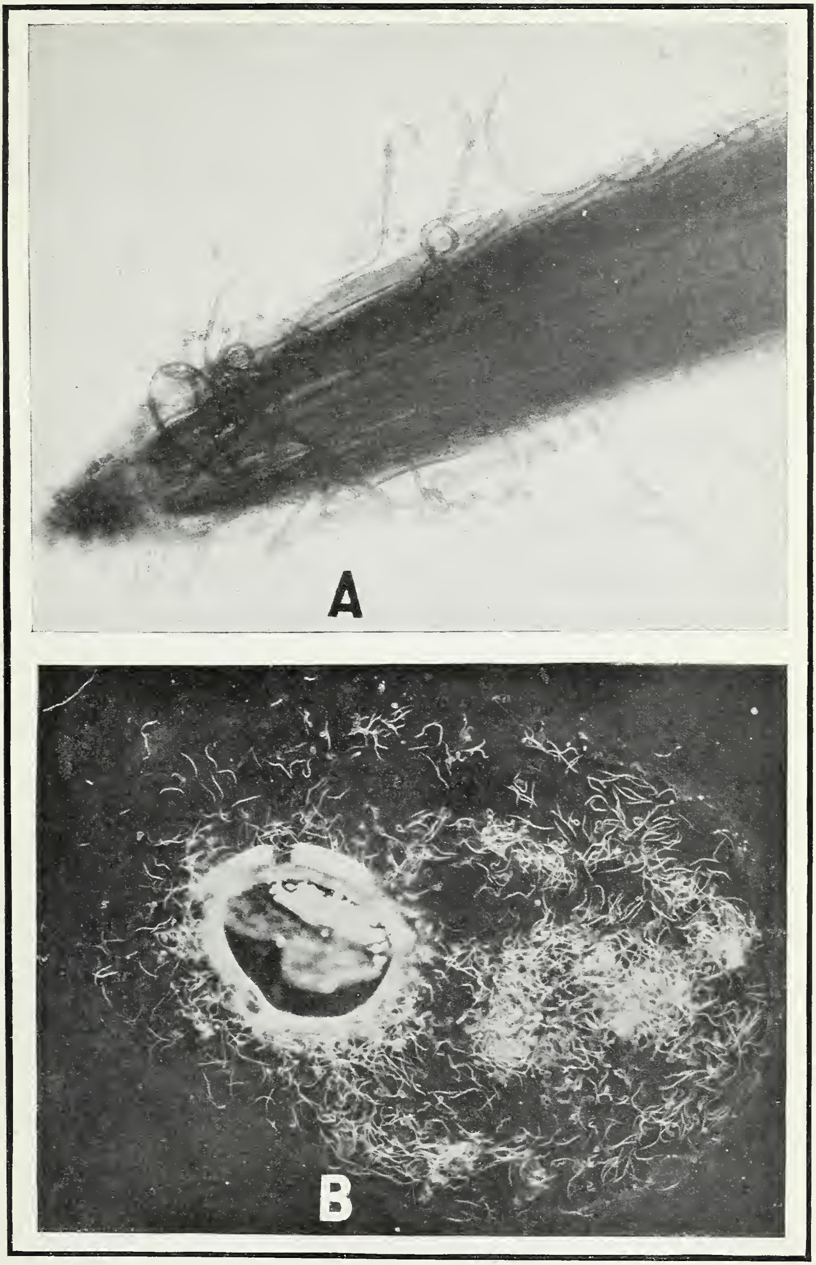
WHEAT SPIKES, INFECTED AND HEALTHY.

A normal head of Leap Prolific wheat between two nematode-infected heads of the same variety. Material collected just before harvest at Remington, Va., June 17, 1918, and painted natural size.



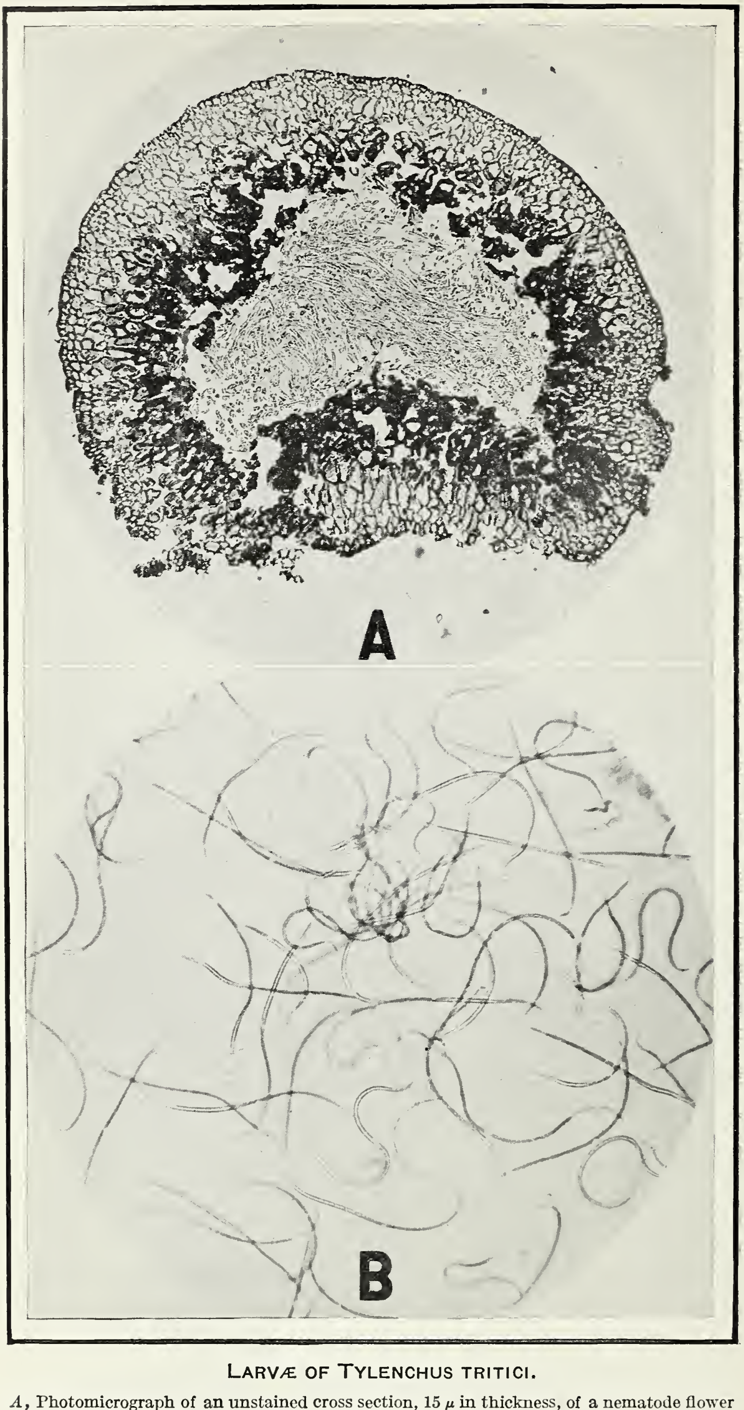
NORMAL KERNELS OF WHEAT WITH NEMATODE GALLS AND OBJECTS FOR WHICH THE LATTER HAVE BEEN MISTAKEN.

FIG. 1 (above).—*A*, Normal kernels of wheat; *B*, dark, smooth nematode galls; *C*, black papillate seeds of cockle; *D*, smutted wheat grains (smut balls); and *E*, bin-burnt kernels. Compare the sound wheat with the nematode galls and note the pronounced difference between the latter and the cockle seed, smutted grains, and the bin-burnt kernels for which the nematode galls are often mistaken. Twice natural size. FIG. 2 (below).—*A*, Unstained, thick cross section of a normal wheat kernel; *B*, cross section of a flower gall filled with a white mass of nematodes, for comparison as to shape, size, and content. × 6.



LARVÆ OF TYLENCHUS TRITICI.

A, Photomicrograph of the root of a wheat seedling growing in a tube of plant-nutrient agar and showing active eellike larvæ accumulating about the root tip. The writer was unable to produce infection through the root, however. × 45. *B*, Masses of milk-white threadlike larvæ photographed while actively moving about in a few drops of water after having escaped from the portion of a cut-open flower gall shown near the center of the photograph. × 6.



LARVÆ OF TYLENCHUS TRITICI.

A, Photomicrograph of an unstained cross section, 15 μ in thickness, of a nematode flower gall, showing larvæ in the center unattached to the inclosing host walls. $\times$ 37. *B*, Photomicrograph of active larvæ from a flower gall moving in a drop of water. Note their transparent anterior ends and their tapering, slightly pointed posterior ends. $\times$ 30.

because they are both caused by related species of nematodes. Tulip-root is induced by another parasitic eelworm, the so-called stem and bulb infesting nematode, *Tylenchus dipsaci* (Kühn) Bastian. This the writer has found in this country on hyacinths, strawberries, clover, and other plants, but not on wheat. Ritzema Bos (29), however, noted the disease on wheat, but described it more in detail as it appears on oats. He notes that on wheat it produces a swelling near the base of the plants, resulting in what he calls a tulip-root appearance. He also states that definite discolored areas result on the stems and leaves. The flowering parts, however, usually are not attacked. As these two closely related wheat diseases differ so strikingly in the way they affect the plant, especially as the so-called "tulip-root" disease does not produce flower galls and the other does, the two diseases are readily distinguishable.

ORIGIN AND DESCRIPTION OF THE GALLS.

The nematode galls may be short and thick or long and slender, and in a dried condition they are nearly always smaller than the wheat kernels. Dwarfed simple galls little more than the size of a pinhead are sometimes found, while those nearly as large as wheat grains are met with frequently. Their size is mainly dependent upon the number of larvæ which enter them, as well as upon the time when and place where the latter penetrate the tissues.

Investigators differ as to the origin and nature of the tissue constituting the flower gall. Davaine (11) thought that the larvæ might enter any portion of the flower and that, therefore, the composition of the resulting gall merely depended upon what tissues were invaded. Haberlandt (15) considered the galls to be largely, if not entirely, of ovarian origin, while Prillieux (28) believed them to be derived only from staminate tissue. Marcinowski (22), after careful and constant macroscopic observations throughout the development of the galls, concluded that they may arise (*a*) from the undifferentiated flower bud, (*b*) at a later stage from staminate tissues which are first differentiated, (*c*) from carpellate tissues which are formed last, and (*d*) from tissue lying between the stamens or between the carpels and stamens. Based on an examination of matured galls as they occur in the wheat spikes, the writer's observations and interpretations agree in the main with those of Marcinowski (22).

Galls within the same flower may be simple; that is, may contain only a single cavity filled with the organism, as shown in Plate V, *B*, and in Plate VI, *A*, or they may be complex. The latter are composed of two or three of the single galls whose walls have grown together and usually show separate interior cavities, as well as deep external furrows, along the lines where the separate units have coalesced. The simple galls are by far the more common and usually occur singly in each flower, although as many as three separate galls have been found in a single flower. Sometimes a young gall may be seen developing in place of one stamen, while the other two stamens and the pistil still appear almost normal. It is rather rare, however, for a normal kernel and one or more galls to develop within the same flower. A young gall is not uncommonly found in place of each of the three stamens, and later these may produce a single trilobed compound gall. In such cases ovarial developments are absent. More commonly, however, a single gall is initiated either in the ovary or the young staminate tissues, and it then usually causes atrophy or nondevelopment in the other reproductive parts of the flower.

Only the reproductive organs or adjacent tissues of the flowers have been found affected, but every flower on a spike may be invaded. It is not uncommon, therefore, to find every flower of a mature spike containing nothing but galls. Heads of wheat well infected with larvæ are often reduced in size, have their glumes sticking out at a greater angle from the rachilla than normal ones, and mature somewhat later than the uninfected spikes. Because of their thickness, the dark maturing galls are often not entirely covered by the glumes, so that they become exposed and thus serve as a conspicuous symptom whereby infected spikes may be readily distinguished in the field (Pl. III).

CAUSE OF THE DISEASE.

The cause of the nematode disease discussed in this paper may be readily seen with the unaided eye when the contents of a gall are placed in clear water. With the aid of a hand lens, the milky white fibrous mass thus liberated, as shown in Plate V, *B*, can be readily observed to consist of thousands of straight or curved threadlike elements, the larvæ of the nematode *Tylenchus tritici* (Steinbuch) Bastian. If watched carefully they will be seen soon to begin active eellike movements. Occasionally among a white mass of larvæ from a mature gall one may see with the naked eye the brown misshapen remains of adult males and females, and with the assistance of a microscope find a few eggs, the stage from which the larvæ developed. In young galls, living adults of both sexes and an abundance of eggs and larvæ may be found.

EGGS.

When viewed from the end the eggs seem to be almost perfectly circular in cross section. In lateral view, which is that most commonly seen (fig. 2), they are elongate, granulated bodies, usually symmetrical in shape. They are usually about twice as long as broad, although individual eggs vary considerably in size even within the same gall. This, however, is not surprising when it is considered that these eggs in a gall may be laid by a number of different females. In three lots of eggs obtained from mature galls collected at widely separated points the writer noted a variation in length from 73 to 140 μ and in width from 33 to 63 μ. An average of all measurements made from material collected in the United States was 38.7 by 85.1 μ, while an equal number of observations taken on eggs from a single lot of galls collected in China gave slightly lower averages, namely, 37.4 by 71 μ. These figures agree very well with those obtained by Bauer (3) in Europe, the dimensions given by him being 28 to 31 by 83 μ (1/800 to 1/900 by 1/300 of an inch). Although the different figures agree in general, there is yet perhaps enough difference to warrant the suggestion that definite strains of the nematode may possibly occur. This suggestion finds further support in data later presented which show what appear to be consistent differences in the dimensions of larvæ collected from various parts of the world. Supporting evidence of possible strains of the organism is also found in certain physiological variations.

FIG. 2.—Lateral view of an old egg, measuring 95 by 40 μ. Camera-lucida drawing. × 190.

The eggs contain dense rounded granules, are semitransparent, and possess a single central light spot, the nucleus. They are covered by a tough, transparent, plastic coat or skin, probably chitinlike in nature, but, unlike the eggs of another endoparasitic nematode, *Heterodera radicicola* (Greef.) Müller, they can not withstand highly unfavorable conditions. This may be due to their not being oviposited in a gelatinous protective secretion, as is the case with the latter, and to their very rapid development. Just how long it takes an egg to develop from the 1-celled state to an active larva is not known. Segmentation, however, is rapid, probably requiring not more than a few days at most, and it ordinarily takes place after oviposition. Within a short time after the egg is deposited it develops into an active larva which pierces the egg coat with its anterior end, escapes, and leaves behind an empty transparent shell.

LARVÆ.

Freshly hatched larvæ are transparent, threadlike animals usually a little more than one-half millimeter in length. Such of their organization as can be observed at low magnification is shown in figure 3. In this first stage, which is of short duration, the larvæ are very delicate, frail, and weak, and ill adapted to withstand unfavorable environmental conditions. They develop quickly into the so-called second larval stage, presumably by going through one or more molts.

It is in this second stage that the larvæ are found as they occur in mature flower galls of wheat, a cross section of one of which is shown in Plate VI, *A*. These larvæ, Plate V, *B*, are slender, cylindrical to spindle shaped animals, slightly blunt at the anterior end, but tapering to a fine point at the posterior end. An outline drawing of a single specimen which measured 884 µ in length is shown at a magnification of 190 diameters in figure 4. In their greatest width the larvæ are from 15 to 20 µ, or about one forty-fifth of the average length, which is from 850 to 890 µ. Based on the measurement of 184 individuals taken from 16 lots of galls collected at as many different localities, the writer found an average length of 869 µ, the extremes being 770 µ and 966 µ. These figures are somewhat smaller than those suggested by Marcinowski (22), who gives the uncertain average larval length of about 1 mm. Dimensions of larvæ from European as well as American material are somewhat larger than those observed by the writer with similar material from China. The average of all measurements of larvæ from a single lot of galls from one point in China was found to be 793 µ, with a maximum range in length from 658 to 910 µ. The dimensions of these larvæ from China are thus seen to average slightly smaller than those examined from other sources. This is similar to the condition pointed out for the eggs from the same material, namely, that they, too, average somewhat smaller. The above indicates that possibly there may exist morphologically different strains or varieties of the organism in different geographical regions.

As is the case with most nematodes parasitic on plants, the structure of the

Fig. 3.

Fig. 4.

Fig. 3.—Young larva, 500 µ in length, which has just emerged from the eggshell. Camera-lucida drawing of lateral view. × 190. For an explanation of the letters *A* to *I*, see figure 4.

Fig. 4.—Old larva, 884 µ in length. Camera-lucida drawing of lateral view. × 190. *A*, Hollow probing spear, through which food is sucked; *B*, muscular esophageal bulb functioning as a pump in sucking food through the spear; *C*, esophageal canal; *D*, posterior esophageal bulb; *E*, digestive system; *F*, nuclei of cells comprising the intestine; *G*, reproductive system at an early stage; *H*, anal opening; *I*, tail.

larvæ of the wheat nematode is comparatively simple. In general, earch larva consists of an outer tube or body covering, within which is a second smaller tube, the digestive tract. The space between the walls of these two tubes is known as the body cavity. Anteriorly, the digestive system opens at the end of the animal, while posteriorly it opens at the anus, or vent, about 50 μ from the point of the tail (fig. 4). A buccal spear (9 to 11 μ long) pointed at the forward end and trilobed at the other, is located at the anterior end of the digestive system (fig. 4, *A*). By means of this hollow spear, which can be exserted and retracted by strong muscles, the larva is able to pierce its egg membranes, to force its way into plant tissues, and by means of the fine canal which extends through the spear to absorb plant juices or other liquid foods. Connected to the base of the spear is the rather slender esophagus, which is about one-quarter the entire body length. About midway of the esophagus is an almost spherical muscular-walled enlargement known as the esophageal bulb (fig. 4, *B*). Near the center of this is a small valve capable of expansion and contraction by the muscular wall of the bulb. By means of a pumplike action of the bulb, liquids are sucked through the spear and esophagus and forced into the intestine. Just back of the esophageal bulb, the esophagus is slender and practically cylindrical, but toward its posterior end it enlarges gradually into another bulb known as the posterior esophageal bulb (fig. 4, *D*). A large light-refractive cell, which functions as a salivary gland, is located near the base of this swelling. This posterior esophageal bulb joins with the much larger tubular intestine, which almost completely fills the posterior three-fourths of the tube of the body wall (fig. 4, *E*). The diameter of the intestine corresponds directly with that of the larva except at its posterior end, where the intestine is contracted into a fine channel, the rectum, which terminates at the anal opening. The intestine contains translucent, granular matter, presumably reserve food, in the form of fatty globules and other materials. The body cavity of the animal not occupied by the digestive system is for the most part practically transparent. Usually a row of refractive nuclei of cells composing the intestinal wall can be plainly seen, and this, together with the half-moon shaped light zone located about midway between the ends of the intestine, distinguishes the larva of this nematode from those of any other species occurring on wheat. No known sexual differentiation occurs in larvæ of the stage just described, although the primordial reproductive organs, consisting of a few hyaline cells located in the half-moon shaped light zone, already mentioned, are always visible. Within the galls, as they mature and dry out, the larvæ, all of which appear to have reached about the same degree of development, become externally desiccated, motionless, and partly coiled. In this condition they are capable of remaining dormant for many years. The writer has recently reactivated in water such larvæ from galls which had been imported along with wheat from Turkestan in 1910. According to Needham (26), a "M. Baher" in 1771 secured vital movement in specimens which the former had sent him 27 years previously, in 1744. During this period the larvæ had been kept in protective galls in the laboratory. When mature galls previously soaked in water are opened, the larvæ upon being freed immediately straighten out with a mechanical movement and later, sometimes within a half hour or occasionally only after a day or more, begin their sluggish eellike vital motions. The two types of movements, though distinctly different and due to different causes, have nevertheless been confused even by trained observers. Dead larvæ may show the former—that is, the mechanical straightening—in a manner very similar to live ones, but only living larvæ show the vital, eellike movements. Very probably, however, "M. Baher" in observing the 27-year-old material referred to above saw both the mechanical and vital movements.

Moistened or submerged in water the larvæ move actively (Pl. V, *B*), but not in the rapid manner so typical of free-living forms. After being held in distilled water at room temperature for more than two months, the writer has observed them still sluggishly moving. During this time the only change noticeable was in transparency, caused undoubtedly by the utilization of reserve food. The translucency increased with the length of time of immersion until finally, at the last observation, they were almost transparent. Liberated in soil under natural conditions, they are capable of living in a free state much longer. Marcinowski (22) concluded from carefully conducted experiments that they could probably live thus in an active condition for seven months or more. In the application of control measures for the wheat nematode, as for the root-knot nematode (*Heterodera radicicola*), use can be made of the fact that these active larvæ will starve to death in a comparatively short period.

Although capable of living free in the soil or elsewhere for several months, the larvæ undergo no development until the host plant is penetrated. By their own eellike locomotion they travel through the soil in search of wheat seedlings, and numerous larvæ finally become located between and within the leaf sheaths near the apical growing points of the culms. They may use the roots as a means of elevating themselves to other parts of the host, but, contrary to what Roffredi (30) and other investigators thought, do not normally penetrate them. The writer was unable to produce root infection by placing large numbers of active larvæ on the surface of sterile tubes of a plant nutrient agar containing wheat seedlings. In such cultures the organisms were observed to move about freely through the transparent medium and to accumulate about the seedling roots, mostly near the root tips, as shown in Plate V, *A*, but no penetration was observed. There was not, however, the marked accumulation about the root tips which the writer (5) has previously observed when pure cultures of the root-knot nematode (*Heterodera radicicola*) were inoculated in similar tubes containing tomato plants. In the latter case there seemed to be some sort of stimulus, possibly chemotactic, attracting the larvæ to the growing point of the root, which many of them entered. Many examinations of wheat seedlings growing in the greenhouse in soil well infested with larvæ of *Tylenchus tritici* showed no root infection.

After having reached the aerial portions of their young host plants some of the wheat nematode larvæ may enter the leaves, but most of them locate in large numbers at the base of and between the leaf sheaths near the terminal growing bud. Here they remain in an ectoparasitic condition, ready to attack the wheat heads as they develop. Thus, contrary to what is commonly thought, the organism of itself travels only a short distance, possibly less than an inch sometimes, in reaching the wheat spikes. The exact manner by which larvæ enter the leaf or flower tissues has never been observed. As there appear to be no natural openings, such as stomata, large enough to permit their entrance, it must be assumed that they get into the inner tissues either by artificial openings or, as is much more likely, by a piercing of the cells with their spearlike anterior ends. At the same time they may possibly secrete some substance to assist in breaking down the cell walls. Only the young, actively growing tissues and cells are invaded.

Wherever larvæ penetrate the leaves, an increase in the size and number of host cells takes place, resulting in a local gall. One or more of these galls may be formed on a single leaf, and they may be located on any portion of the latter, depending upon the place and time of larval penetration. The larvæ usually enter only the young leaves which are entirely inclosed within the older leaf sheaths. Their entrance somewhat retards and prevents normal development, causing the leaves to grow unevenly and to become wrinkled, rolled, and distorted. These symptoms are illustrated in Plate II, *A* and *B*, which in addition shows a small gall on the edge of the youngest leaf. This gall, which is typical, is about 4 mm. long, 3 mm. wide by 2 mm. thick, and has lost its normal green color, being almost white. Upon teasing it apart 25 of the nematodes were found in various stages of development, including sexually mature females almost ready to lay eggs. This, however, is an unusually large number to find in a leaf gall.

Ordinarily a wrinkling and rolling of leaves on infected seedlings is not accompanied by the production of leaf galls, and one may search in vain to find larvæ within these wrinkled leaves. In just what manner the parasite induces this wrinkling without remaining in the leaf is not known. It may possibly be the result of the extraction of food substances in the form of plant juices from the leaves by the larvæ when the leaves are young and inclosed in the outer leaf sheaths.

While the leaves are sometimes invaded, it usually is the embryonic flowers into which the larvæ penetrate and produce the most conspicuous signs of the disease. Either before or just after differentiation of the terminal flower buds larvæ pierce the outer layers of cells, reaching the inner tissues in numbers usually larger than 2 and seldom more than 25. There they stimulate cell growth and gall production. Within each gall a cavity is formed, and in this the larvæ develop into sexually mature individuals, males and females, in about equal numbers. These then copulate, and before the galls are mature the females lay thousands of eggs. The latter then hatch, and the new larvæ go into dormancy as the galls mature.

ADULTS.

As soon as the larvæ have entered the host tissues they rapidly develop within the cavity of the growing gall into sexually mature females and males which, as shown at equal magnification in figures 5 and 6, are markedly different morphologically. They are of sufficient size to be plainly visible to the naked eye.

FEMALES.

Mature females vary considerably in length. The writer has found them in young green flower galls only 3.42 millimeters long, while Marcinowski's greatest measurement of length is 5.23 millimeters. Doubtless even wider variations than these occur. The average length seems to be about 4 millimeters. Their greatest width, though larger in proportion to their length than that of the larvæ, is also subject to great variations within the same individual. Young females may not measure more than 168 μ, while old females when well filled with eggs are much wider.

In general, the sexually differentiated females still remain spindle shaped, tapering gradually from the middle toward each end, but being somewhat thicker and fuller anteriorly (fig. 5). The head end terminates bluntly, while the tail end is distinctly pointed. Morphologically, the esophageal portion of the digestive system of the adult is not greatly different from that of the larva except as to size. Behind the posterior swelling of the esophagus, however, there are marked differences both in the intestine and the reproductive system of the adult as compared with those of the larva. In the young female the intestine, occuping a large part of the entire body cavity, becomes proportionately small as the reproductive system increases, so that in old individuals most of the body space is taken up by the egg-producing organ. The intestine is finally pressed closely against the body wall throughout its anterior half, while in the posterior portion it

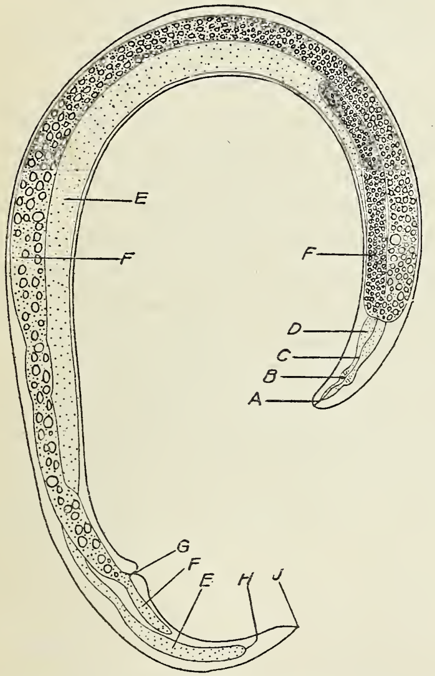


FIG. 5.—Lateral view of young female 1.95 mm. in length. Camera-lucida drawing. × 95. For an explanation of the letters *A* to *J*, see figure 6.

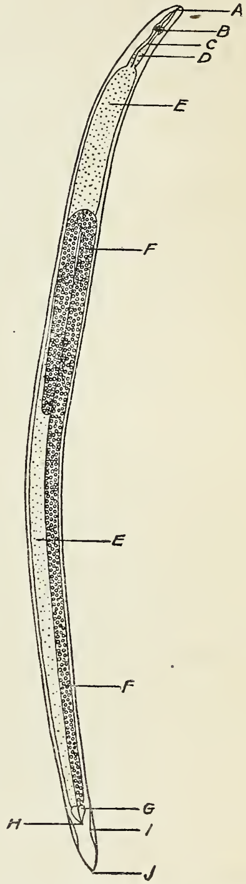


FIG. 6.—Ventral view of young male 1.36 mm. in length. Camera-lucida drawing magnified × 95. *A*, Spear; *B*, anterior esophageal bulb; *C*, esophageal canal; *D*, posterior esophageal bulb; *E*, digestive system; *F*, reproductive system; *G*, spicula in male and vulva in female; *H*, anus; *I*, bursa of male; *J*, tail.

gradually lessens in diameter and barely touches the wall. It contains at first granular bodies as well as liquids, and the entire contents appear yellowish brown in color. In old females, however,

151572°—20——3

the intestine is almost colorless, transparent, and void of a large part of its granular food material.

The reproductive system, composed of two branches, opens at the vulva, which is situated about one-eighth to one twenty-seventh of the body length forward from the tail end. The fertile branch which extends in front of the vulva is at first glandular and saclike for a short distance. It then becomes smaller and continues as a fine tube of uniform diameter to near the middle of the organism. This tubelike portion, the uterus, is usually filled with many fertilized eggs in various stages of development, and in old females may contain hatched larvæ. The uterus expands into a vesicular-shaped portion, presumably the receptaculum seminis, where fertilization takes place, and the latter opens into the large end of the ovary, which contains mature ova. Usually the ovary folds twice, once near the anterior end of the intestine and again just forward of the middle of the animal, and terminates in a small blind end filled with rudimentary eggs. Back of the vulva there is a short, bag-shaped, glandular, sterile branch of the genital organs, the function of which is not understood.

As females mature they become tightly coiled and motionless, except for a slight motion of the head. Although still capable of being bent, they will, if straightened out and released, quickly recoil into a shape somewhat similar to that of a watch spring.

Egg laying begins soon after the galls are formed and continues for several weeks, during which time a single female may lay more than 2,000 eggs. As an average of about six or seven females are developed in each flower gall, the latter usually contains about 15,000 eggs or an equal number of larvæ into which the eggs soon develop. By actual count the writer has found 11,573 and 18,051 larvæ in what were selected as two medium-sized galls, while Dr. N. A. Cobb informs him that he has found as many as 90,000 in a very large gall. Like larvæ of the first stage, adult females as well as males are incapable of withstanding unfavorable conditions of temperature and moisture.

MALES.

As may be seen from figure 6, the adult male differs conspicuously from the female. It is much shorter, measuring from about 2 to 2.5 millimeters in length, or approximately one-half that of the female, and is more slender, its maximum width being from one-twentieth to one-thirtieth of the length. The anterior end is broader and not as rounded as in the female, and slightly in front of the pointed tail end it possesses a curved transparent wing, the bursa, with which females may be held. Near the center of this bursa is located the opening of both the intestine and the reproductive organs. The latter extends as a tube of about uniform diameter nearly up to the esophagus, folds back for a short distance, and terminates in a closed end. This tube is usually well filled with developing or mature spermatozoa, which with the aid of a copulating organ, the spicula, located near the sexual opening, may be ejected during coition. Unlike the intestine of the female and similar to that of the larvæ, the intestine of the male extends, largely undisturbed by the reproductive system, from the base of the esophagus nearly to the anus as a tube of uniform diameter.

The male is capable of movement during practically its entire life, retains its larval shape, and probably copulates only once. Sluggishly moving males are often found in galls which contain only the shapeless disorganized remains of females. As has been stated, males and females occur in about equal numbers.

EFFECT OF HIGH TEMPERATURES ON THE LARVÆ.

LARVÆ INSIDE OF THE GALLS.

In order to determine the maximum thermal death point of the larvæ, the writer has treated a large number of galls with hot water at various temperatures. The results of these tests are presented in Tables I and II. Galls soaked several hours or days in unheated tap water were used in obtaining the data given in Table I, while in Table II the data were obtained from treating the dry material. In all cases the same method was used in applying the hot-water tests. It consisted in submerging lots of two to six galls for the desired length of time in a large volume of water, the temperature of which did not vary during the course of the experiment more than 1 degree above or below that required, and usually varied less than half a degree in either direction. Following the treatment, each gall was placed in distilled water in a Syracuse watch glass, then carefully opened with sterilized instruments, and the larval contents microscopically examined repeatedly for several days afterward. In the first few experiments larvæ were discarded after having been under observation for one day subsequent to treatment, but it was soon discovered that some of the organisms showed signs of life at a later date, so in all succeeding experiments the larvæ were observed over a period of two to nine days. A total lack of movement of the larvæ in combination with the coagulation of their internal granular contents was taken as the criterion of death. The largest number of larvæ found to be living while they were under observation was roughly estimated in the percentage of the average number of individuals (about 1,500) contained in each gall. All the nematode galls used in these tests were from the same source, namely, a collection made on a farm near Union Mills, Va.

WATER-SOAKED GALLS.

A glance at the results presented in Table I, obtained from treating galls previously soaked, shows that the larvæ are only slightly affected by an immersion of as much as 15 minutes in water at 46°, 48°, and 50° C., respectively. They are completely killed, however, when exposed for 30 minutes or more in water heated to 50° C. Almost identical results secured by the writer after subjecting another parasitic nematode, *Heterodera radicicola*, to the same treatments suggest that other plant-attacking nematodes may respond similarly.

TABLE I.—*Effect of hot-water treatment on larvæ of the wheat nematode inclosed in wet galls.*

Lot.	Number of galls, each containing an average of more than 1,500 larvæ.	Hot-water treatment.			Extent of observation after treatment (days).	Larvæ active (per cent).							
						In each gall at last examination.						Average—	
		Date, 1918.	Temperature (° C.).	Duration.		1	2	3	4	5	6	In each lot.	In each treatment.
				Minutes.									
No. 15	6	Mar. 18	46	5	2	90	50	90	80	95	80	80.83	80.83
No. 14	5	...do	46	10	2	75	10	1	75	75	....	47.2	47.20
No. 13a	6	...do	46	15	2	90	2	10	90	60	100	58.66	58.66
No. 13	6	...do	48	5	2	90	90	90	85	60	80	82.5	82.50
No. 12	6	...do	48	10	2	90	90	90	90	50	85	82.5	82.50
No. 11a	6	...do	48	15	2	85	85	75	50	70	75	73.33	73.33
No. 9	6	...do	50	5	3	75	65	40	85	85	75	70.83	70.83
No. 8	6	...do	50	10	4	10	2	4	70	75	30	31.83	31.83
No. 17	6	Mar. 22	50	15	3	90	85	90	85	95	10	75.83	75.83
No. 18	6	...do	50	20	3	90	70	95	85	95	45	80	40.00
No. 2	6	Mar. 13	50	20	3	0	0	0	0	0	0	0	......
No. 19	6	Mar. 22	50	25	3	85	75	80	65	90	30	70.83	70.83
No. 23	5	Mar. 25	50	30	4	0	0	0	0	0	0	0	0
No. 24	5	...do	50	35	4	0	0	0	0	0	0	0	0
No. 25	2	...do	50	40	4	0	0	....	....	....	....	0	0
No. 26	5	...do	50	45	4	0	0	0	0	0	....	0	0
No. 11	6	Mar. 18	52	5	4	45	30	40	50	30	60	44.16	44.16
No. 10	6	...do	52	10	7	0	40	35	1	1	60	22.83	18.1
No. 20	6	Mar. 22	52	10	4	0	0	.26	80	0	0	13.37	
No. 21	6	...do	52	15	4	0	0	.13	.5	.26	85	14.3	
No. 27	5	Mar. 25	52	15	5	0	0	.26	0	0	....	.51	3.7
No. 40	6	Apr. 6	52	15	6	0	0	0	0	0	0	0	
No. 44	5	Apr. 16	52	15	6	0	0	0	0	0	0	0	
No. 22	6	Mar. 22	52	20	4	0	0	0	0	0	0	0	
No. 28	5	Mar. 25	52	20	4	0	0	0	0	0	....	0	
No. 39	6	Apr. 6	52	20	6	0	0	0	0	0	0	0	0
No. 45	5	Apr. 16	52	20	6	0	0	0	0	0	....	0	
No. 29	3	Mar. 25	52	25	4	0	0	0	....	....	....	0	0
No. 38	5	Apr. 6	52	25	6	0	0	0	0	0	0	0	
No. 1	6	Mar. 13	54	10	3	0	0	0	0	0	0	0	
No. 30	5	Mar. 25	54	10	5	0	0	0	0	.49	....	.08	
No. 35	6	Apr. 6	54	10	6	0	0	0	0	0	0	0	.02
No. 41	5	Apr. 16	54	10	6	0	0	0	0	0	....	0	
No. 5	6	Mar. 13	54	15	3	0	0	0	0	0	0	0	
No. 31	5	Mar. 25	54	15	9	0	0	0	0	.2	....	.04	.01
No. 36	6	Apr. 6	54	15	6	0	0	0	0	0	0	0	
No. 42	5	Apr. 16	54	15	6	0	0	0	0	0	....	0	
No. 37	6	Apr. 6	54	20	6	0	0	0	0	0	0	0	......
No. 43	5	Apr. 16	54	20	6	0	0	0	0	0	....	0	0
No. 34	5	Apr. 4	56	5	8	0	0	0	0	0	....	0	0
No. 32	5	...do	56	10	8	0	0	0	0	0	....	0	0
No. 33	5	...do	56	15	8	0	0	0	0	0	....	0	0
No. 3	6	Mar. 13	58	5	3	0	0	0	0	0	0	0	0
No. 4	6	...do	58	10	3	0	0	0	0	0	0	0	0
No. 6	6	...do	60	5	3	0	0	0	0	0	0	0	0
Check	6	...do	(1)	2	2	95	95	95	95	95	95	95	95
				Days.									
Do	6	Mar. 20	(1)	8	8	95	95	95	95	95	95	95	95

[1] Room temperature.

By increasing the temperature of the water to 52° C., all larvæ become motionless and lifeless after an exposure lasting 20 minutes. Exposures for a shorter time than this considerably reduced but did not entirely stop larval activity. In water at 54° C. the larvæ were practically all killed in 10 minutes, although there were six larvæ from one gall, or 0.02 per cent of the series, which at the end of this time showed faint signs of life. Likewise, when treated at the same temperature for 15 minutes, 0.01 per cent, or three larvæ, were found sluggishly moving. In both cases the fractional percentages of nematodes not killed are small enough to come within the expected limits of error, and in addition the faintly moving larvæ appeared so weakened as never to be able to parasitize the host. Thus, at least for all practicable purposes, these two treatments seem absolutely effective. No living larvæ were found in galls immersed longer than 15 minutes in water at 54° C., and the same was true if they were exposed to the liquid at 56°, 58°, and 60° C., respectively, for 5 minutes or more.

In the check experiments, 95 per cent of the unheated larvæ in the soaked and dry galls remained alive throughout the investigations.

It is both interesting and important to note that the hot-water treatments found effective for the nematode should so closely correspond to those which have been used successfully in controlling the loose smut of wheat caused by *Ustilago tritici*. Humphrey and Potter (17) recommend treating water-soaked wheat for 10 to 15 minutes in water heated to a temperature ranging from 52° to 57° C. (125° to 135° F.) to control this smut. Because of this fact, a single operation should suffice for both the nematode disease and the smut.

DRY GALLS.

Larvæ within dry unsoaked galls are much less affected by a given hot-water treatment than those contained in galls previously moistened throughout. No doubt this is due to the longer time required to raise the internal temperature of the dry gall to the thermal death point of the nematode. As shown in Table II, 29 per cent of the larvæ survived a hot-water treatment of 50° C. for 30 minutes, while 36, 24, and 55 per cent lived after immersions in water at 52° C. for 20 minutes, 54° for 10 minutes, and 56° for 5 minutes, respectively, all of which are treatments to which the larvæ in soaked galls succumbed. Only by increasing the time of exposure beyond that specified were the various temperatures entirely effective in killing the nematodes.

The figures obtained by the writer on the hot-water treatments differ materially from those of Davaine (11) and Marcinowski (22), who state that larvæ in galls are not killed by an immersion for 10 to 12 minutes in water at a temperature of 54° to 56° C. If the statements of these investigators, however, were supported by experimental data these were not presented, and furthermore, they failed to mention the important point as to whether they were treating dry galls or those that previously had been soaked in water.

TABLE II.—*Effect of hot-water treatment on larvæ of the wheat nematode inclosed in dry galls.*

Lot.	Number of galls, each containing an average of more than 1,500 larvæ.	Hot-water treatment.			Extent of observation after treatment (days).	Larvæ active (per cent).							
		Date, 1918.	Temperature (° C.).	Duration.		In each gall at last examination.						Average—	
						1	2	3	4	5	6	In each lot.	In each treatment.
				Minutes.									
No. 1	6	Mar. 13	50	10	2	0	0	0	15	5	0	3.3	3.3
No. 8	5	June 5	50	15	4	35	35	90	30	90	....	56	56
No. 2	6	Mar. 13	50	20	2	0	75	0	2	0	0	8.5	36.7
No. 9	5	June 5	50	20	4	40	40	90	75	80	....	65	
No. 24	5	June 17	50	30	3	60	.5	50	25	10	....	29	29
No. 25	5	...do.....	50	35	3	10	20	0	40	0	....	14	14
No. 26	5	...do.....	50	40	3	0	15	8	0	5	....	5.6	5.6
No. 27	5	...do.....	50	45	3	0	.06	15	20	0	....	7.1	7.1
No. 10	5	June 5	52	10	4	35	80	75	45	90	....	65	65
No. 11	5	...do.....	52	15	4	10	65	0	10	0	....	17	17
No. 12	5	...do.....	52	20	4	90	20	0	70	0	....	36	36
No. 28	5	June 17	52	25	4	1	20	15	55	40	....	26.2	26.2
No. 13	5	June 5	54	10	4	0	0	60	0	60	....	24	24
No. 5	6	Mar. 13	54	15	2	0	0	0	0	0	....	0	6.5
No. 14	5	June 5	54	15	4	0	0	35	10	20	....	13	
No. 15	5	...do.....	54	20	4	30	0	0	0	0	....	6	6
No. 16	5	...do.....	56	5	4	0	90	45	60	80	....	55	55
No. 17	5	...do.....	56	10	4	0	75	40	0	0	....	21	21
No. 18	5	...do.....	56	15	4	0	0	0	0	0	....	0	.1
No. 29	5	June 17	56	15	3	0	0	0	.2	0	....	.2	
No. 3	6	Mar. 13	58	5	2	0	5	0	75	0	30	18.6	29.8
No. 19	5	June 5	58	5	4	50	0	65	90	0	....	41	
No. 4	6	Mar. 13	58	10	2	0	80	.06	0	0	0	16	28.5
No. 20	5	June 5	58	10	4	50	0	65	90	0	....	41	
No. 6	6	Mar. 13	60	5	2	50	75	2	3	80	75	47.5	38.7
No. 21	5	June 5	60	5	4	70	0	80	0	0	....	30	
No. 22	5	...do.....	60	10	4	0	0	0	0	0	....	0	0
				Days.									
Check	6	Mar. 13	(1)	3	3	95	95	95	95	95	....	95	95
Do	5	June 5	(1)	4	4	95	95	95	95	95	....	95	

[1] Room temperature.

LARVÆ OUTSIDE OF THE GALLS.

Active larvæ liberated from galls were exposed to hot water in the following manner in order to ascertain their response to high temperatures. By means of a glass dropper about 300 nematode larvæ were transferred to diminutive bags of silk and then placed in a large receptacle of water of the desired temperature. After a specified time the bags were removed, the contents of each immediately emptied into distilled water in a Syracuse watch glass, and the larvæ examined microscopically on each of several days following the treatment. The highest percentage of nematodes found living at any one observation was recorded and is presented in Table III.

TABLE III.—*Effect of hot-water treatment on active larvæ of the wheat nematode taken from galls.*

Experiment.	Number of lots of larvæ of about 300 each.	Hot-water treatment.			Extent of observation (days).	Larvæ living (per cent).		
		Date.	Temperature (° C.).	Duration.		By lots. 1	By lots. 2	Average, by treatments.
				Minutes.				
No. 9	1	Mar. 27	48	3	4	85	....	85
No. 10	1	...do.....	48	5	4	90	....	90
No. 11	1	...do.....	48	6	4	50	....	50
No. 6	1	...do.....	49	3	4	90	....	90
No. 7	1	...do.....	49	6	4	50	....	50
No. 8	1	...do.....	49	10	4	20	....	20
No. 3	1	...do.....	50	3	4	75	....	75
No. 1-2	2	Mar. 26	50	5	4	50	60	
No. 27-28	2	Apr. 29	50	5	4	.6	4	19.7
No. 45-46	2	May 7	50	5	4	3	1.5	
No. 4	1	Mar. 27	50	6	4	15	....	15
No. 5	1	...do.....	50	10	4	5	....	
No. 18	1	Apr. 15	50	10	6	0	....	.8
No. 29-30	2	Apr. 29	50	10	4	0	0	
No. 47-48	2	May 7	50	10	4	0	0	
No. 19	1	Apr. 15	50	15	6	0	....	
No. 31-32	2	Apr. 29	50	15	4	0	0	0
No. 49-50	2	May 7	50	15	4	0	0	
No. 20	1	Apr. 15	50	20	6	0	....	0
No. 12	1	Mar. 27	51	3	4	20	....	
No. 33-34	2	Apr. 30	51	3	4	2	4	5.8
No. 51-52	2	May 7	51	3	4	2	1	
No. 13	1	Mar. 27	51	6	4	1	....	
No. 21	1	Apr. 15	51	6	6	0	....	.7
No. 35-36	2	Apr. 30	51	6	4	2	1	
No. 53-54	2	May 7	51	6	4	0	0	
No. 14	1	Mar. 27	51	10	4	0	....	
No. 22	1	Apr. 15	51	10	6	0	....	0
No. 37-38	2	Apr. 30	51	10	4	0	0	
No. 55-56	2	May 7	51	10	4	0	0	
No. 23	1	Apr. 15	51	15	6	0	....	0
No. 15	1	Mar. 27	52	3	4	0	....	
No. 24	1	Apr. 15	52	3	6	0	....	2.2
No. 39-40	2	Apr. 30	52	3	4	10	1	
No. 57-58	2	May 7	52	3	4	1	.6	
No. 16	1	Mar. 27	52	6	4	0	....	
No. 25	1	Apr. 15	52	6	6	0	....	.3
No. 41-42	2	Apr. 30	52	6	4	.16	2	
No. 59-60	2	May 7	52	6	4	0	0	
No. 17	1	Mar. 27	52	10	4	0	....	
No. 26	1	Apr. 15	52	10	6	0	....	0
No. 43-44	2	Apr. 30	52	10	4	0	0	
No. 61-62	2	May 7	52	10	4	0	0	
				Days.				
Check	2	Mar. 27 Apr. 29 May 7	(1)	4	4 6 6	95 95 95	95 95 95	95

[1] Room temperature.

It is to be noted that neither the submersions at 48° nor 49° C. were of sufficient duration to kill all the larvæ, even though the number remaining alive was somewhat below that of the untreated lots held as a check. Treatments with water at 50° to 52° C. gave uniform results; that is, the percentage of larvæ surviving varied directly with the length of immersion. For example, at 50° C., 19.7, 15, and 0.8 per cent survived after exposures of 5, 6, and 10 minutes, respectively, while none lived where the exposure lasted for 15 minutes. Comparable results were obtained at higher temperatures, with all larvæ succumbing at 51° and 52° C. in 10 minutes. It is interesting to note that the minimum length of time required to kill all larvæ in these experiments at 50° and 52° C., namely, 15 and 10 minutes, respectively, is exactly half that consumed in accomplishing an identical result with the nematodes inclosed in galls which were soaked in water previous to treatment. Thus, the data as a whole in Table III show conclusively that the active parasites are much more sensitive to high temperatures outside of the protective galls than inside of them.

EFFECT OF CHEMICALS ON THE LARVÆ.

As adequate and dependable data regarding the response of larvæ to some of the chemicals more commonly used as sterilizing and disinfecting agents were not available, the writer has carried out a large number of experiments which were planned to obtain such data. In these experiments, as in those with hot water, both the free larvæ removed from galls and the protected larvæ inclosed in galls were treated. Experiments with the protected larvæ will be discussed first.

LARVÆ IN GALLS.

The method of procedure followed in subjecting the nematode galls to the chemicals and in determining the effect of the latter on the larvæ was essentially the same as that used in the hot-water investigations; that is, water-soaked galls were submerged in the solution for the desired length of time, then washed in water several times, and each opened in distilled water in a Syracuse watch glass. The larval contents were subsequently examined microscopically daily for several days to determine the effect of the treatment. The average number of larvæ from each lot of galls surviving the treatment, as well as other data, is recorded in Table IV.

By a study of Table IV it will be seen that unheated formaldehyde at strengths of 1:640, 1:320, 1:160, 1:100, and 1:80 produced no marked effect on the larvæ within two or four hours. Submerged in a 1:50 solution, however, for two hours only 2 per cent of the larvæ in the galls remained alive, and when kept in this same strength of the chemical for four hours none survived. Likewise, all larvæ were killed when the galls were treated in 4 per cent formaldehyde for two and four hours, respectively. A 1:320 strength of the chemical at a temperature of 50° and 52° C. killed less than half the larvæ when the galls were treated for one, two, and four minutes. It was thought that these hot solutions might be effective in eliminating the nematode, since Melhus (23) controlled oat smut by applying a 1:320 strength at temperatures of 45°, 50°, and 55° C. for one minute. In the galls kept in tap water for four days and used as a check on those treated with formaldehyde, 95 per cent of the nematodes were alive.

TABLE IV.—*Effect of formaldehyde, mercuric chlorid, copper sulphate, and sulphuric acid on nematode larvæ inclosed in water-soaked galls.*

Chemical and temperature.	Treatment.			Number of galls treated.	Average larvæ living in each lot (per cent).
	Strength.	Exposure.	Date, 1918.		
		Hours.			
Formaldehyde: At room temperature (about 21° C.)	1: 640	2	Mar. 22	3	65
		4	...do....	3	90
	1: 320	2	...do....	3	73
		4	...do....	2	85
	1: 160	2	...do....	5	64
		4	...do....	3	90
	1: 100	2	Apr. 3	5	79
	1: 100	4	...do....	5	54
	1: 80	2	Mar. 22	3	72
		4	...do....	3	82
	1: 50	2	Apr. 3	5	2
	1: 50	4	...do....	5	0
	1: 25	2	...do....	5	0
		4	...do....	5	0
		Minutes.			
At 50° C.	1: 320	1	Apr. 17	5	59
		2	...do....	5	61
		4	...do....	5	70
At 52° C.	1: 320	1	...do....	5	80
		2	...do....	5	63
		4	...do....	5	54
		Days.			
At room temperature (check)	Tap water	4	Mar. 22	5	95
			Apr. 3	3	95
		Hours.			
Mercuric chlorid	1: 1,000	3	Oct. 19	3	63
		4	...do....	3	58
		5	...do....	3	28
		6	...do....	3	15
		7	June 4	6	0
		17	June 3	6	0
	2: 1,000	3	Oct. 19	2	57
		4	...do....	2	62
		5	...do....	2	37
		6	...do....	2	0
		7	June 4	6	0
		17	June 3	6	0
	4: 1,000	3	Oct. 19	3	40
		4	...do....	3	15
		5	...do....	3	0
		6	...do....	3	0
		7	June 4	6	0
		17	June 3	6	0
	10: 1,000	3	Oct. 19	3	46
		4	...do....	3	0
		5	...do....	3	0
		6	...do....	3	0
		7	June 4	6	0
		17	June 3	6	0
Copper sulphate	5 per cent	7	Apr. 4	5	78
		17	...do....	5	22
	10 per cent	7	...do....	5	10
		17	...do....	5	4
	15 per cent	7	...do....	5	4
		17	...do....	5	1
Sulphuric acid	½ per cent	7	...do....	5	74
		17	...do....	5	2
	1 per cent	7	...do....	5	5
		17	...do....	5	0
	1½ per cent	7	...do....	5	0
		17	...do....	5	0
		Days.			
Check	Tap water	5	..do....	5	95
			Oct. 19	5	90
			June 3	5	95

It appears, therefore, from a study of the results presented in Table IV that the nematode in protected galls does not readily succumb to formaldehyde and will survive treatments with this chemical which seriously impair the germination of the wheat.

In Table IV also are given the data secured by treating soaked galls with mercuric chlorid, copper sulphate, and sulphuric acid. As can be seen, these results are comparable to those obtained with formaldehyde, namely, that the nematode is strikingly resistant to the chemicals and that treatments with these chemicals which are not injurious to the wheat fail to kill the larvæ.

LARVÆ OUTSIDE OF THE GALLS.

The method of treating unprotected larvæ outside of the galls consisted in placing the actively moving nematodes, usually those removed from a single water-soaked gall, in the solution in a Syracuse watch glass. From the time the larvæ were put into the dish they were kept under continual microscopical observation for several hours, or a day, until after all signs of life had disappeared or until the treatment was discontinued. At the end of the experiment the percentage of larvæ living was noted, and this, with other data, is given in Table V.

TABLE V.—*Effect of various chemicals on unprotected larvæ of the wheat nematode outside of galls.*

Chemical.	Treatment.				Number of lots of larvæ.	Larvæ living (per cent).
	Strength.	Exposure.		Date.		
		Hours.	*Minutes.*			
Formaldehyde	1: 240	4	55	May 31	3	0
		5	5	June 1	3	0
	1: 120	3	15	May 31	3	0
		3	5	June 1	3	0
	1: 100		36	May 14	2	0
			45	May 17	3	0
	1: 80		32	May 31	3	0
			25	June 1	3	0
	1: 50		46	May 14	2	0
			50	May 17	3	0
	1: 25		14	May 14	2	0
			17	May 17	3	0
Mercuric chlorid	1: 1,000	4	25	May 31	3	0
		4	35	June 1	3	0
	2: 1,000	2	27	May 31	3	0
		2	55	June 1	3	0
	4: 1,000	2	0	May 31	3	0
		2	30	June 1	3	0
	10:1,000	2	0	...do....	3	0
Copper sulphate	5 per cent	6	30	May 16	2	85
		8	35	June 1	3	5
		17	0	May 17	2	0
	10 per cent	6	15	May 16	3	60
		7	15	June 1	3	0
		17	0	May 17	3	0
	15 per cent	6	15	May 16	3	20
		7	45	June 1	3	0
		17	0	May 17	3	0
Sulphuric acid	½ per cent	3	20	...do....	3	60
		7	0	June 1	3	0
	1 per cent	3	5	May 17	3	40
		5	25	June 1	3	0
	1½ per cent	3	5	May 17	3	.001
		4	50	June 1	3	1
		Days.				
Check	Tap water	5		May 14	3	95
				May 31	3	95

A study of Table V shows that the larvæ are extremely resistant to the toxic action of the chemicals, living 4 hours and 55 minutes in formaldehyde of 1:240 strength and 4 hours and 25 minutes in mercuric chlorid of 1:1,000 strength. Submerged for 6½ hours in a 5 per cent solution of copper sulphate, 85 per cent of the larvæ remained alive; and immersion for 3⅓ hours in sulphuric acid of 0.5 per cent strength failed to kill 60 per cent of them. These few figures are sufficient to indicate the impracticability of using these chemicals for disinfecting seed.

By comparing the figures in Table IV with those in Table V it will be seen that a considerably longer time was required for the chemicals to kill the larvæ when they were inclosed in galls than when they were not.

OVERWINTERING OF THE PARASITES.

It is in the second larval stage that the organism lives from one season to the next. But just where most of these larvæ overwinter outside is an unsettled question about which there is no uniformity of opinion among the investigators who have studied the problem. Some of the earlier investigators, including Roffredi (30) and Henslow (16), were of the opinion that the larvæ overwintered in the protective galls which shattered out of the wheat heads at harvest time and dropped to the ground or else during the fall were sown in the soil along with the seed. According to these earlier workers the larvæ did not escape from the galls into the soil and infect the wheat plants until spring. On the other hand, Davaine (11) in 1857 concluded from his experiments that infection takes place in the fall when the nematodes are freed from the galls and that the larvæ overwinter in the young wheat plants. Since that time this view had been generally held by scientific workers until 1909, when Marcinowski (22) deduced from rather extensive experiments that most of the larvæ remain in the galls throughout the winter, although a few may escape at almost any time during the fall or winter and either live free in the soil or locate in the seedlings. It will thus be seen that there is no general agreement as to the manner in which the parasite passes the winter in the field.

The writer has tentatively concluded from preliminary observations on field experiments which are still under way that most of the larvæ get out of the galls in the fall and overwinter either in a free state in the soil or as an ectoparasite in the seedlings. Evidence supporting this view was found in January, 1919, when a large number of nematode-infected seedlings were observed in an experimental field plat. These infected seedlings occurred in rows of wheat which were grown from wheat seed planted October 11, 1918, in uninfested soil. Sown along with the seed was an equal volume of unopened nematode galls. It seems probable, therefore, from the large number of seedlings infected and from the many parasites found in some of the plants, that most of the larvæ must have escaped from the galls before January. It is not safe, however, to draw general conclusions from such limited observations, and the manner in which the larvæ live through the winter may depend upon the seasonal conditions occurring in the fall. Most likely the nematodes are liberated from the galls when favorable moisture and temperature conditions exist, regardless of whether this be in the fall, winter, or spring. That the larvæ, either within or without the gall, may withstand extreme cold is indicated by the laboratory experiments of Davaine (11), who subjected them without injury to a temperature of 15° to 18° C. below zero for five hours. The writer has kept them embedded in ice for several days and on reactivating them in water observed no injurious effect of the treatment. Indeed, the larvæ began their movements while the water was barely above the freezing point.

HOST PLANTS.

Investigators disagree as to the host range of *Tylenchus tritici*. Most of them, however, maintain that it is a highly specialized parasite normally attacking, to any appreciable extent, only wheat.

Both Roffredi (30) and Marcinowski (22) were able to produce only small, imperfect flower galls of barley (*Hordeum vulgare*) and rye (*Secale cereale*) by artificial inoculation with larvæ. In a similar manner more pronounced infection was secured in spelt (*Triticum spelta*), although in this plant the number and size of the galls fell much below that of wheat (*Triticum vulgare*) subjected to like treatment. Also, under natural conditions, spelt is reported to have been slightly diseased. Henslow (16), without adducing convincing evidence, states that he obtained with difficulty a slight infection of oats (*Avena sativa*), rye, and barley.

During the season of 1918–19, the writer, in cooperation with A. G. Johnson and R. W. Leukel (9), induced an abundant infection of emmer (*Triticum dicoccum*), rye, and spelt, and a slight infection of oats. These crops were grown in a previously uninfested field and were inoculated by sowing in certain rows seed mixed with nematode-containing galls. In other rows seed infested with free nematodes was planted.

In the case of rye, spelt, and emmer, flower galls of about the size of those produced on wheat occurred on numerous spikes of these plants. In oats only a few small flower galls were found in a limited number of plants. Similar experiments on barley gave only negative results.

Thus, from the rather limited data available on the subject it is evident that the nematode may parasitize cereal grasses other than wheat, although apparently not to so great a degree. Further careful observations in the field and more adequate cross-inoculation experiments are necessary, however, in order definitely to determine the relation of the nematode to these cereals.

On many wild grasses, flower galls due to species of nematodes closely related to and by many believed to be identical with *Tylenchus tritici* have been found by both European and American observers. Bessey (4) in 1905, for example, reported that he had found such galls in grasses of the genera Chaetochloa, Agropyron, Elymus, Calamagrostis, and Trisetum collected in Texas, Oregon, and Alaska, but was unable to determine whether any of them were induced by an eelworm identical with the wheat nematode. Some investigators have given the causal organism occurring on the various grasses specific names, such as *Tylenchus agrostidis*, *T. graminearum*, and *T. phalaridis*, depending largely upon the host attacked, and sometimes in the brief inadequate description of these parasites the writers have pointed out minor differences between them and *Tylenchus tritici*. On morphological grounds alone these described differences are not sufficient to warrant separating them from the wheat nematode, and for this reason Dujardin (13), Diesing (12), and Bastian (2) in their helminthological monographs have considered them identical with *Tylenchus tritici*. Whether the gall-producing nematodes discovered on different grasses are physiologically alike in parasitism can, of course, be determined only by cross-inoculation experiments, a thing which the above-mentioned investigators apparently did not attempt. Marcinowski (22), however, in an experiment covering two years, failed to induce flower infection of many grasses with *Tylenchus tritici*. Each plant during the first year was inoculated with the larvæ of several hundred wheat galls and during the next year was again treated with the larval content of about 300 galls. The grasses used in the experiment were, for the most part, those on which galls had been reported. They are as follows:

Agrostis capillaris, A. stolonifera, A. canina.
Bromus erectus, B. pratensis, B. secalinus.
Alopecurus geniculatus.
Festuca ovina, F. pratensis, F. vulgaris.
Holcus lanatus.
Poa annua, P. pratensis.
Phleum bohmeri, P. pratense.

Although suspicious symptoms appeared in the leaves during the second year on *Festuca vulgaris*, *Poa annua*, and *Alopecurus geniculatus*, not a single gall was found on any of these hosts, and the fruit matured normally.

In examining many of the common grasses, especially *Bromus secalinus* and *Dactylis glomerata*, growing in and near severely diseased fields of wheat in Virginia, the writer has been unable to find nematode galls of either the flower or leaf. The evidence at hand, therefore, seems to indicate that the nematode on wheat is not identical with forms producing flower galls on wild grasses.

Not infrequently nematode galls located only on the leaves of grasses have been reported. Some of these are caused by organisms morphologically similar to and by some investigators also thought to be identical with *Tylenchus tritici*. The writer has found such leaf infections particularly abundant on *Calamagrostis canadensis* in Wisconsin, but these are caused by a species of Tylenchus different morphologically from the wheat nematode. In general appearance they resemble somewhat the uredinia of certain rusts, for which they have been mistaken. As a rule, they are small, yellow, ellipsoid swellings, about 5 mm. long, 2 mm. wide, and 2 mm. thick, which involve the entire thickness of the leaf at the affected point, causing it to protrude slightly from the upper and lower surfaces. Their long axis extends in a direction parallel to the midrib, and they may occur on any portion of the leaf. Grasses so affected have been observed many times in both America and Europe. As in the case of the nematodes causing flower galls on various grasses, it is not possible, on account of the brevity of descriptions, to determine positively whether all the nematodes in the leaf galls of grasses differ from *Tylenchus tritici*. On the whole, it seems rather unlikely that any of them are identical with the wheat organism, since the latter is primarily a parasite of the inflorescence and only occasionally produces leaf galls.

An interesting and important question is whether identical organisms are responsible for the leaf and the flower galls of the wild grasses, on some of which both types of attack occur. In the case of wheat, the writer has shown that the two kinds of galls may be induced by *Tylenchus tritici*. Davaine (11) obtained similar results and Kieffer (20) also noted definite leaf swellings which contained a species of Tylenchus very likely identical with *T. tritici*, but Marcinowski (22) was unable to find leaf galls on wheat.

From what has been said it is evident that considerable investigation is needed to extend our knowledge of the relation between *Tylenchus tritici* and other nematodes occurring on grasses.

METHODS OF SPREADING THE PARASITES.

Only in its second larval stage does the parasite constitute a source of new infection. All other stages of the life cycle are more or less transitory, unable to withstand unfavorable conditions, and unable to live or develop for any appreciable time outside the host plant.

It is evident that the larvæ are most frequently introduced into new localities while contained in the galls. The latter may be transported along with seed wheat in which they are mixed, or in soil, or by many other means. If conditions are unfavorable for the larvæ to escape from these galls, the parasite is able to remain in a lethargic dried state for many years and afterwards to produce the disease. The organism may also be transferred long distances outside of the protective galls, in which case it probably could not remain alive and active for more than one year unless a host plant were found. In this free state the larvæ are most likely to be carried in soil, which is practically the only place where they normally occur outside the host. Although the spreading of free larvæ may take place, it is, of course, almost entirely by means of those contained in galls that the organism reaches widely separated areas. In the latter condition it has been repeatedly introduced into this country with importations of seed wheat. In all probability such introductions would have been entirely prevented or long delayed if careful inspection of all shipments had been made. In the future every precaution should be taken to prevent its further importation.

Once introduced into a locality the larvæ are capable of traveling by their own movements only very limited distances in the soil. Haberlandt (15) states that he obtained infection in seedlings which were growing in the ground 20 cm. distant from an unopened gall. The writer has found abundant infection in seedlings growing 30 cm. away from unopened galls which were buried a few inches below the surface of the ground. When it is considered that this movement must have taken place within a short time, probably less than a month, the rate of travel for so small an animal is fairly rapid. Probably during the course of a growing season, in which infection may take place at any time if young plants are available, the organism might be able to cover several feet or even a few yards. It is, however, not through their own efforts that these larvæ mainly are spread. Many other means are at their disposal. Either within or without the galls, they may be distributed by running water or water resulting from heavy rains. They will not drown after months of submersion, and as drying for a long period does not kill them they may be carried alive in small particles of desiccated soil in various ways, such as on the feet of men and animals, and by agricultural implements. Fortunately, there usually is but little tramping over fields while they contain growing wheat, so that this is an unimportant means of spreading the parasite except when no crop is present.

Of course, the chief manner of spreading the parasite within a locality is by the sowing of seed wheat which is mixed with nematode galls. As pointed out later, it is possible to free the seed by a simple method, yet it is the part of wisdom to sow clean seed which has been grown in uninfected areas.

METHODS OF CONTROL.

Fortunately, the nematode disease appears to lend itself fairly readily to control measures. At least this has been the experience of European agriculturists, and there seems to be no reason why the malady should not respond to proper combative methods in this country. Indeed, the measures employed are so obvious and apparently so effective that very little attention has been given to this feature of the disease by research men, no doubt for the reasons given. Successful methods of control are given below, based on the etiology of the disease as presented and on limited field observations by the writer and investigators in England and continental Europe.

CLEAN SEED.

To prevent the occurrence of the malady it is absolutely essential that clean wheat unmixed with nematode galls be secured for seed purposes. The disease may be detected most readily at harvest time or after thrashing. If dark, hard galls, containing a white mass of nematodes, are found in the wheat, such grain should not be used for seed unless no other is available. Instead, a supply grown in localities in which the disease does not occur should be obtained.

However, if clean seed is not obtainable, the nematode galls may be separated from the sound wheat by the so-called salt-brine method devised by Johnson and Vaughn (18) for removing ergot from rye. Essentially it consists in pouring the infected seed slowly into a water solution containing 20 per cent of common salt (NaCl) and at the same time stirring vigorously. Because of their lower specific gravity, the nematode galls, light kernels, smutted grains, the seeds of some weeds and grasses, and other foreign material will float, while the sound grain will sink. After removing the galls and other floating matter from the surface of the solution, pour off the latter and rinse the grain in water immediately. After being freed from nematode galls the grain should be spread out to dry, after which it is ready for planting. With Dr. A. G. Johnson, the writer has successfully employed this salt-brine method, separating and removing every gall from several bushels of wheat which contained more than 50 per cent of the injurious galls. The method is not only entirely effective and inexpensive, but also readily adaptable for operations involving small or large quantities of grain. The germinating quality of the grain is not impaired by the treatment.

The writer has also separated nematode galls from small quantities of diseased wheat by another method, which requires considerable care and effort for its successful execution. It is carried out by releasing water under pressure at the bottom of a receptacle which contains the infected wheat and stirring and roiling vigorously at the same time. If the water is released through a hollow rod attached to a hose the rod may also be used in stirring the grain. With such treatment the nematode galls and other light material will rise to the surface and may be easily removed. Whether this water method would be effective for use where large quantities of grain are to be separated has not been determined.

In removing the galls by either of these means there is little likelihood that seed so cleansed will bear any living larvæ on the surfaces of the kernels. Only rarely do the larvæ escape from galls which are held under the usual storage conditions, and any that might escape would most likely be killed by the usual rubbing together of the kernels as the grain is handled. On drying out, the larvæ become stiff and brittle and are very easily broken. However, in order to eliminate the possibility of transmitting any source of infection along with the sound kernels the cleaned seed can be treated with hot water at a temperature of 51° to 52° C. for 10 minutes, a treatment which the writer has found to kill unprotected larvæ. According to Humphrey and Potter (17) this treatment is also sufficient for controlling loose smut (*Ustilago tritici*). If the hot-water bath is to be given, it should be applied directly after the wheat has been separated from the galls.

Although the methods just described would seem to obviate the necessity of any other means of disinfecting the grain, another means of accomplishing equally good results is available. It consists of soaking the gall-mixed seed for about an hour in unheated water and immediately immersing it for 20 or more minutes in water held at a temperature of 52° C. Equally effective has been found either an immersion of the soaked seed for 15 minutes in water maintained at 54°, or for 10 minutes at 56° C. The writer has never been able to find living nematodes within galls given any of these treatments, although these observations are apparently at variance with those of Marcinowski (22), and Davaine (11), who report that they were unable to kill the organism by immersing the galls in water at a temperature of 54° to 56° C. for 10 to 12 minutes. Doubtless they did not previously soak the infected seed. This supposition would account for the difference in results, as it takes a much longer time to raise the internal temperature of dry galls or seeds than that of those which are wet.

The various chemicals ordinarily used for disinfecting purposes, such as formaldehyde, mercuric chlorid, and copper sulphate, failed to kill the nematodes when used in strengths which did not injure the seed.

CROP ROTATION.

Crop rotation, as well as the use of nematode-free seed, is essential in controlling the disease, as the indications are that the parasite may live in the protective gall for one or more years in soil in which no congenial host grows, and at the end of that time constitute a source of infection. If infested land be planted for two or preferably for three consecutive years to nonsusceptible crops, most or all of the nematodes will be eliminated. Although these nematodes are capable of remaining alive, but inactive, in a dried, stiffened state for a number of years, under the usual field conditions the temperature and moisture are such that they become active during the warmer months, and in this motile stage they will starve in less than a year provided no susceptible plant is available. Almost all of the active larvæ will have starved in the soil during the first year after a diseased crop has been harvested, but it is advisable not to plant a susceptible crop on infested land until after two or more years. In England, where a 2 to 3 year rotation of crops, including wheat, is now commonly practiced, Ormerod (27) reports that no difficulty in controlling the disease has been met with if uninfected seed wheat is sown. Marcinowski (22) made a similar report of conditions in Germany.

Until our knowledge concerning the range of the host plants of *Tylenchus tritici* is more complete, it is highly inadvisable to grow not only any variety of wheat, oats, rye, emmer, or spelt but also any of the closely related grasses on land that is to be freed from nematodes. So far as known, all strains or varieties of wheat are equally susceptible, none having been reported as resistant. All wild grasses, especially those on which flower galls caused by nematodes have been found, should be scrupulously kept off the land, as some of them may be susceptible to the wheat nematode. These grasses, as well as weeds, may be more easily kept down if the rotation crops are planted in rows so as to permit clean cultivation. As there are no plants outside of the grass family on which *Tylenchus tritici* has been reported, several presumably immune crops exist which can be used in rotations designed to control the disease. Every precaution against reinfesting the soil should be taken if success in controlling the nematodes by crop rotation is to be obtained. Spreading the pest by those means already pointed out should be prevented.

Other methods of controlling the parasite have been proposed, but because of their inefficiency or impracticability they have not been adopted. Kühn's (21) catch-crop method, once thought to be a practicable means of controlling the sugar-beet nematode (*Heterodera schachtii*) has been proposed. It consists of growing a susceptible plant, in this case wheat, on the land, allowing it to become well infected, and then destroying the young plants at the proper time to catch the largest number of nematodes. Then by removing the crop from the land many of the parasites are eliminated. The method is ineffective because only a portion of the nematodes free in the soil enter the seedlings, and it is also impracticable because of the cost involved. Deep plowing is a more successful means of reducing the disease, but this by itself is not adequate to eliminate the nematodes. The deeper the larvæ are turned under the soil below a depth of a few centimeters, the less opportunity they have for reaching and infecting plants. Marcinowski (22) obtained a higher percentage of infection when the nematodes were placed 3 centimeters below the surface of the soil than at any other depth, and only a small number were able to reach the wheat seedlings buried at a depth of 30 centimeters.

SUMMARY.

A serious disease of wheat known in Europe since 1743 and due to the nematode *Tylenchus tritici* (Steinbuch) Bastian has been recently found causing considerable damage in certain parts of the United States, particularly in Virginia.

Reports in the literature and an examination of specimens received from various foreign countries show that the malady is almost world-wide in distribution, having been discovered in all continents except Africa. Within the United States it is known to occur at present in California, Virginia, West Virginia, Maryland, and Georgia, and other States also may be found to be infested.

Samples of harvested wheat collected in Virginia contained from less than 1 per cent to more than 50 per cent of diseased kernels by count, and as much as 40 per cent damage by it to some fields of wheat in the same State has been observed.

On seedlings the disease usually causes a decided wrinkling and rolling or distortion of the young leaves, which, if severely attacked, turn yellow, wilt, and die. Occasionally such leaves have one or more small light-colored galls, which may be located on any portion of the leaf and contain the causal organism. Roots are not directly affected, nor do the stems of infected plants near the surface of the ground become swollen, as in the case of tulip-root, another disease of wheat, caused by the nematode *Tylenchus dipsaci*, which infects stems and crowns. It is on the maturing spikes that the malady produces the most conspicuous symptoms. As a rule, infected heads

retain their green color longer than normal spikes. They are reduced in length and have their glumes spread out at greater angles than uninfected spikes. In place of normal kernels the glumes contain dark, hard galls, which are shorter and frequently thicker than wheat grains and which may be seen sometimes partly exposed in diseased spikes. Because of a general similarity, these galls often have been mistaken for the seed of cockle (*Agrostemma githago*), a weed which occurs commonly in wheat fields, for kernels affected by stinking smut due to *Tilletia tritici*, and for bin-burnt wheat caused by overheating the stored grain.

The disease is caused by a minute nematode, *Tylenchus tritici* (Steinbuch) Bastian, the larval stage of which in great numbers may be seen with the unaided eye when a gall is teased apart in water. These larvæ in mass appear milky white in color. Individually they are threadlike animals, nearly 1 millimeter in length and capable of an eellike movement. Under favorable conditions of moisture and temperature they escape from the galls which have fallen to the ground or have been sown along with wheat seed, and by their own movement reach the young seedlings. They finally become located between the leaf sheaths near the terminal stem bud and in this region are passively elevated to the inflorescence. Occasionally they penetrate the leaves and induce small swellings, but usually they enter only the embryonic flowers, which develop into galls instead of kernels. In these places the larvæ, after several metamorphoses, develop into about equal numbers of male and female adults, the former about 2 mm. in length and the latter from 3 to 5 mm. long. Each female may lay more than 2,000 eggs. Measuring approximately 37 μ wide by 85 μ long, these eggs are elongate, ellipsoidal, granular bodies, which in a short time after oviposition develop into larvæ and in this manner complete the life history of the organism. At the maturity of the plant the larvæ within the gall become inactive, dried, and motionless, in which lethargic condition they can remain alive for many years.

The larvæ inclosed in galls were killed by an immersion in water at temperatures of 50°, 52°, 54°, and 56° C. for 30, 20, 10, and 5 minutes, respectively, provided the galls were thoroughly moistened before the treatment. Much longer immersions at the same temperatures are necessary to kill them if the galls are dry when treated. Free from the protective gall, the larvæ succumb to temperatures of 50° and 52° C. in 15 and 10 minutes, respectively; that is, in half the time required to kill them within the galls. Larvæ either inside or outside the protective gall are highly resistant to the toxic action of formaldehyde, mercuric chlorid, copper sulphate, and sulphuric acid. They live after long submersion in concentrations of these chemicals, which in a short time are injurious to seed wheat.

The nematode appears to be primarily a parasite of wheat. To a less extent it parasitizes rye, oats, spelt, and emmer, and it has been reported on barley.

The malady is most commonly spread by means of seed wheat containing nematode galls. It may be distributed in various other ways, such as by the feet of men and animals and by agricultural implements to which dirt containing viable larvæ may cling. Surface water resulting from rains often transports the parasite from an infested to an uninfested field.

The disease may be controlled by the use of nematode-free seed in combination with the employment of a 2 to 3 year crop rotation and the application of proper sanitary precautions. Uninfected wheat for planting should be secured, if possible, from localities where the disease does not occur. Nematode-free seed, however, may be obtained from diseased grain by floating off the galls in a 20 per cent salt solution and then treating the remaining wheat with water at a temperature of 50° to 52° C. for 10 minutes. Diseased seed can also be freed from nematodes without removing the galls by immersing them in water at a temperature of 54° to 56° C. for 10 to 12 minutes. By keeping wheat off infested land for two or, better, for three years, all or most of the nematodes will have starved, so that the subsequent wheat crop will not be appreciably damaged. Every precaution should be taken to prevent the reintroduction of nematodes to land which is being rotated to control the disease.

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