WOUND INFECTION AND TISSUE INVASION BY PLASMODIOPHORA BRASSICAE[1]

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INTRODUCTION

Our earliest knowledge of the parasite concerned with the clubroot disease of Cruciferae goes back to the the studies of Woronin, which began in 1873. His first published account of the disease appeared in 1875 (*6*)[3], and the complete description of the organism (*Plasmodiophora brassicae* Wor.) and its intimate relation to the host was published in 1878 (*7*). Woronin was unable to find the myxamoebae actually pressing their way into the host cells, but implied that initial penetration took place through root hairs and epidermal cells of the primary root. He was of the opinion that migration of the plasmodia within the host tissue took place directly through the cell walls, and that a decided irritation of the invaded tissues extended well beyond those cells actually occupied by the parasite. The next important study of the parasite and its relation to its host was made by Nawaschin (*4*), who worked on diseased material sent to him by Woronin. He started his cytological study in 1893, but made little progress until 1897, when he adopted the fixing and staining methods of Flemming. He did not observe the passage of the parasite from cell to cell within the host tissues, and believed, therefore, that migration of the plasmodia by direct penetration of the cell walls did not take place. He concluded that the organism spread through the tissue by the multiplication of the invaded cells and the consequent division of the plasmodia between the daughter cells.

Lutman (*3*) made no attempt to ascertain the manner of initial infection, but believed that the parasite entered either through the young thin-walled epidermal cells or the root hairs. He reported migration by division of infected cells and figured direct penetration of the cell walls of the host tissue. Chupp (*1*) was of the opinion that direct infection occurred only through root hairs, and that direct penetration of the epidermal cells of the cabbage root did not take place. He confirmed Lutman's observation of migration of plasmodia from cell to cell by penetration of the cell walls.

Kunkel (*2*) secured infection of older root and lower stem by the application of macerated clubs to the unwounded surface. He found no evidence that primary infection took place through wounds, but was of the opinion that invasion was not necessarily confined to the

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[3] Reference is made by number (italic) to Literature Cited, p. 623.

root hairs or the primary tissues of the young rootlets. He pointed out that the plasmodia spread in the root and rootlike tissues from the point of original infection to adjacent tissues, but that they migrate rather promptly through the periderm (the primary cortex having disappeared in tissues of this age) to the cambium, where they stimulate great activity of the host cells. After reaching the cambium the plasmodia no longer penetrate tne other tissues with equal readiness, but follow the path of least resistance. In the cambium the myxamoebae increase their area of attack, since in the subsequent meristematic activity the parasite is distributed to many cells. Kunkel believed that migration in the host tissue is accomplished primarily through direct penetration of cell walls.

This paper is a report of further study of infection of the hypocotyl and stem of cabbage and related plants, and the relation of the clubroot organism to the tissues of these organs. Certain observations on root invasion are reported for comparative purposes.

MATERIALS AND METHODS

Yellows-resistant cabbage plants were employed because of the presence of *Fusarium conglutinans* Woll. in the clubroot-infested soil. Plants grown in clubroot-free greenhouse soil until approximately 90 days old were used for the inoculation experiments, which were usually conducted in a greenhouse bench. Infested soil was secured from a badly diseased field in Kenosha County, Wis. Diseased cabbage roots were also collected from this field and stored in sand until needed. Healthy cabbage roots were secure from clubroot-free soil and stored in the same manner. Certain inoculation series were conducted in naturally infested soil in the field.

Tissues were prepared for histological study by placing them in killing fluid immediately after removal from the plant. Formalin-acetic-alcohol fixative was used in the early part of the study. It was found necessary later to use a killing agent in which the tissue hardness would not be altered. A chromo-acetic-formalin solution consisting of 100 cc of 1-percent chromic acid in water, 4 cc of glacial acetic acid, and 50 cc of formalin was found to be most satisfactory when made up fresh for each fixation. When there was need of fixing relatively large pieces of tissue, incomplete fixation was avoided by subjecting the material immediately to a partial vacuum for a period of 2 to 4 hours, depending on the size of the fragments being fixed. Zirkle's (*8*) normal butyl-alcohol method of dehydration, clearing, and infiltration of tissue with paraffin was used. Embedding was done in paraffin with a melting point of about 55° C. Microtome sections were cut in thickness varying from 10μ to 15μ; a few were cut 20μ thick. A combination of safranine (1 percent in 50-percent alcohol) and fast green (1 percent in absolute alcohol) was found to be the most satisfactory stain for the structures studied. In addition, Delafield's haematoxylin was used with fair success.

EXPERIMENTAL RESULTS

INFECTION STUDIES WITH CABBAGE

During a study of soil treatment for the control of cabbage clubroot, the writer used, in one series, greenhouse-grown cabbage plants in which the internodes had become longer than usual. In setting

these plants into naturally infested soil the cotyledons and 3 or 4 of the lower leaves were removed, and the roots were placed so deeply in the soil that several of the stem internodes were below the surface. After approximately 4 weeks it was found that not only had the roots become infected but that galls had developed at the injured cotyledonary plate and on the stem at points where the leaf petioles had been removed. A careful macroscopical study of the infected tissue showed that the disease on the stems was confined to those portions which had been injured by the removal of the cotyledons or the leaf petioles. The portions that were not injured (internodes) were quite free from infection. On the stem the hyperplastic tissue took the shape of a spheroid gall, while in the roots the clubs were typically spindle-shaped (fig. 1).

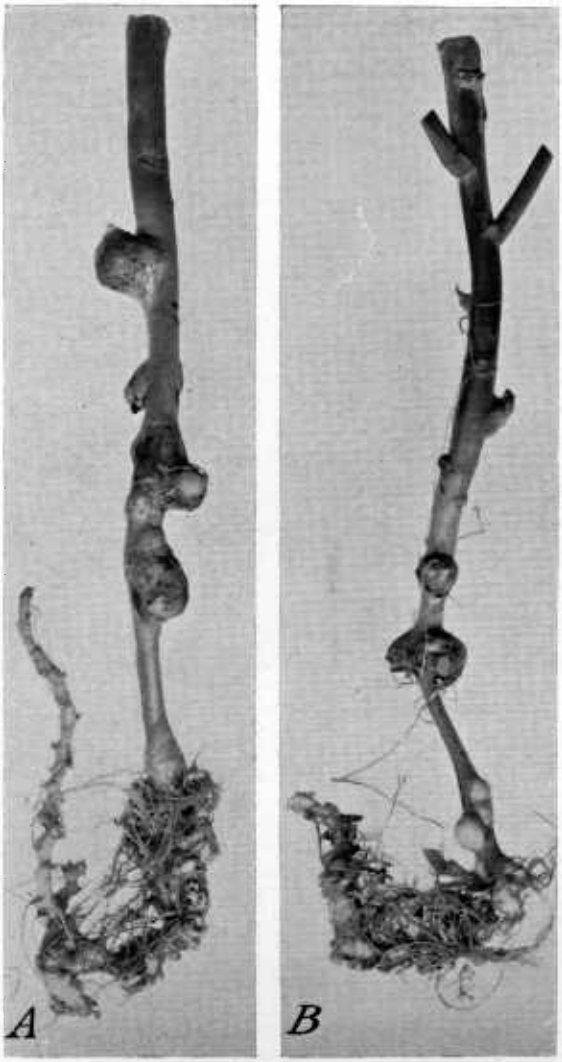

FIGURE 1.--Cabbage plants which were wounded at the lower leaf nodes and the cotyledonary node by removing the petioles at the stem, and transplanted immediately to clubroot-infested soil. The plants were 3 months old when wounded and were removed and photographed 1 month later. Note the spheroid galls which developed at the wounded nodes.

Since the foregoing observation indicated that infection of the cabbage stem occurred through wounds rather than by direct penetration of uninjured tissue as reported by Kunkel (*2*), experiments were outlined to clarify this point. Plants were divided into two groups. In 1 group the lower 4 leaves were removed by cutting the petioles at the juncture with the stem. In the second group the lower four leaves were removed by cutting the petiole 1 cm away from the stem. In the latter group the normal abscission layer at the juncture of stem and petiole formed and the remainder of the petiole dropped off in a

few days. The plants were set into clubroot-infested soil as soon as the petioles were removed. The stems were submerged so that the soil line was well above the fourth node. About 4 weeks was allowed for each experiment, since it had been determined previously that abundant clubs appeared on roots within this period.

In those plants in which the wounded stem tissue was exposed to the infested soil, infection occurred generally and the characteristic

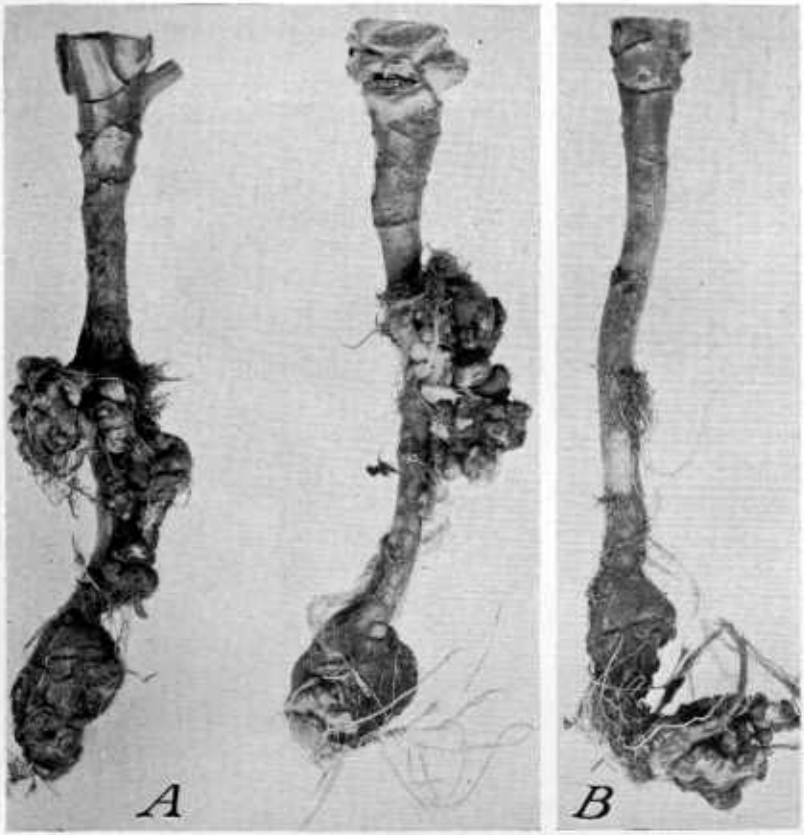

FIGURE 2.—Infection of cabbage stems in the field following injuries inflicted by the removal of leaf petioles. These plants remained in the soil for approximately 2 months. *A*, Clubs produced at the wounded nodes of the stem; secondary clubs developed on the spheroid galls as a result of infection of adventitious roots. *B*, Plant in which the stem was not injured before planting; the stem remained free from clubroot infection. Note that the root clubs developed on the injured as well as on the uninjured plants.

spheroid galls observed earlier were formed at the nodes. In those plants in which the normal abscission layer formed at the nodes only an exceptional gall developed. Histological examination of these exceptional cases showed that infection had occurred as a result of the rupture of tissue by adventive roots. In both sets of plants the usual root clubs developed, showing that there was abundance of inoculum in the soil and that the roots were infected readily. The experiment was repeated twice in the field with similar results. Figure 2 shows typical stem galls on plants wounded at the node as they were transplanted to naturally infested field soil, contrasted with a plant in the

same series in which the normal abscission layer at the node developed after transplanting.

In order to determine the relation of wound-cork formation to club-root infection, 20 plants were set aside after the removal of the leaf petioles at the nodes. After 5 days on the greenhouse bench, during which time cork formation began, they were transplanted to infested soil in the usual way. An equal number of plants were wounded at the node and planted immediately. At the end of 4 weeks, there was no evidence of infection at the nodes of plants which had been held 5 days after leaf removal, although galls were numerous on those freshly wounded at the time of transplanting. This experiment was repeated with similar results.

The possibility of infection of the internodes by way of needle punctures was next studied. Only plants free from adventive roots were used, and care was taken to avoid any other injury. Leaf petioles were severed 1 cm from the stem and needle punctures made midway between nodes in the first four internodes. The plants were set into infested soil as in previous experiments. Infection took place in the first internodes in 80 percent of the wounded areas, but in no case was it observed in the upper internodes. In the latter the punctured areas were discolored and the surrounding tissue had collapsed. The general outline of the hypertrophy of the first internode was intermediate between the spindle produced in rootlike structures and the spheroid galls produced at the stem nodes (fig. 3, *D*). Spindle formation usually did not extend more than 1 to 2 cm above or below the point of infection. In the region of injury, the gall formation was very pronounced, increasing the diameter of the cortex as much as five times the normal.

The next experiments were with the hypocotyl, which, as will be pointed out in greater detail later in the paper, is predominantly root-like in structure. Each plant was wounded with a single needle puncture about equidistant from the taproot and the cotyledonary node and set at once in infested soil. Plants which were not thus wounded served as checks. After 4 weeks the roots of all plants were generally infected. The hypocotyls became infected, however, only in those plants which had received needle wounds. The hypertrophies in this series in contrast to the spheroid galls formed at the stem nodes were typical spindles similar to those found generally on the roots (fig. 3, *A*). The unwounded hypocotyls remained free from infection (fig. 3, *B*). This experiment was repeated several times with similar results both in the greenhouse and in the field.

Kunkel (*2*) reported spindle-shaped clubs as a result of artificial inoculation of the lower stem, although the histological sections of his material indicate that he included hypocotyl in his studies. He inoculated by applying infectious debris (diseased root tissue) to a given point on the stem. When this method was used by the writer, formation of spindles occurred when the inoculum was applied to the hypocotyl or the first nodes (fig. 3, *E* and *F*), but was unsuccessful when applied to the second or higher nodes. When infested soil instead of debris was applied at a given focus no infection occurred unless needle wounds were supplied.

Kunkel's results led him to believe that infection of the stem occurred readily without the presence of wounds. As will be shown later in the paper, the application to stem or hypocotyl of decaying root tissue,

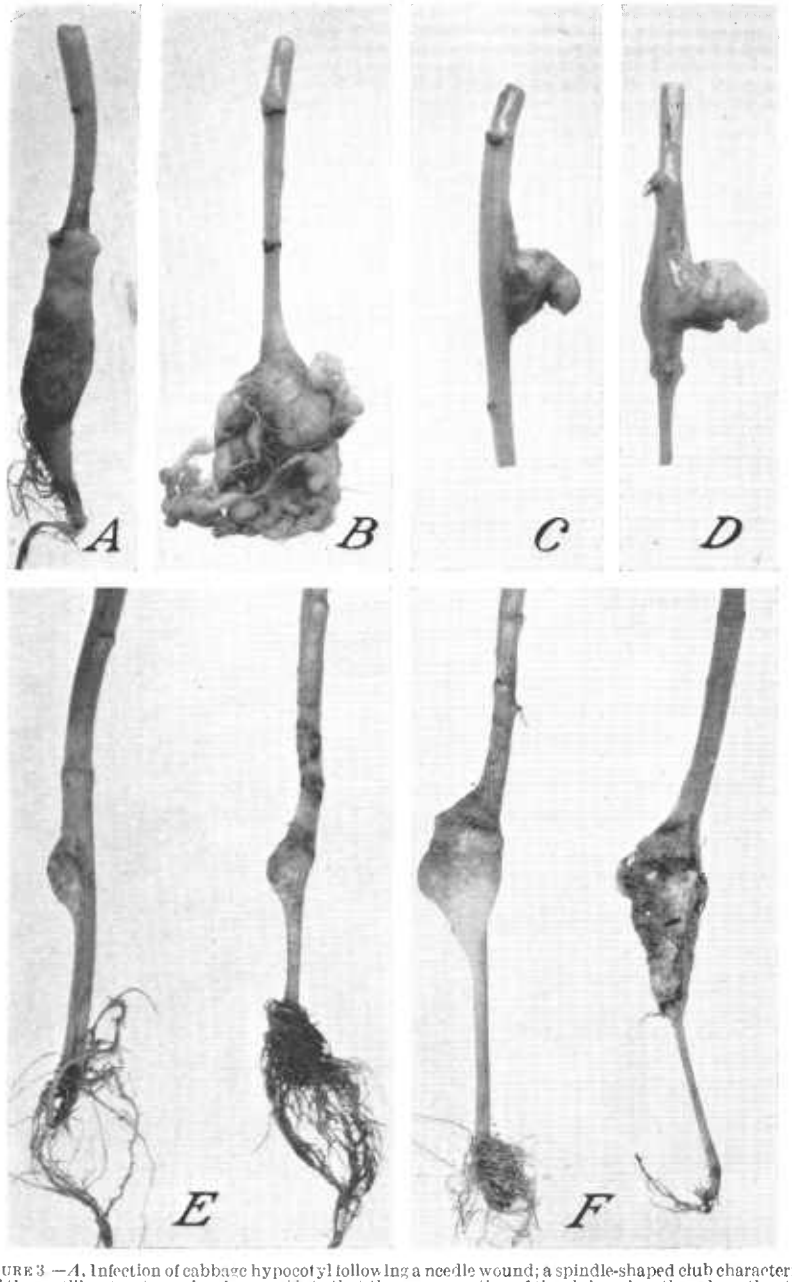

FIGURE 3.—*A*, Infection of cabbage hypocotyl following a needle wound; a spindle-shaped club characteristic of the rootlike structures developed. Note that the upper portion of the club ends rather abruptly at the cotyledonary plate. *B*, Root clubs formed on cabbage in naturally infested soil. The hypocotyl was not wounded and remained uninfected. *C*, Spheroid gall formed on an injured stem node. *D*, Infection of the first internode following a needle wound. The hypertrophy is intermediate between the spindle and gall types. *E* and *F*, Cabbage plants infected following inoculation by Kunkel's method. The spindle-shaped clubs are thickest at the point to which the infectious material was applied. *E*, Photographed 20 days after the inoculum was applied, and *F*, after 30 days.

whether clubroot-infested or not, has a corrosive effect upon the tissue and provides an unnatural opening for the parasite. The writer's experiments show conclusively that infection of the stem and hypocotyl of cabbage by the clubroot organism does not occur through the unwounded surface. However, the parasite may invade readily through the injured stem nodes and through wounds on the hypocotyl or first internode. The hypertrophy on the stem is spheroid, while on the hypocotyl it tends to be spindle-shaped as in the root proper.

INFECTION STUDIES WITH RADISH AND TURNIP

Several varieties of radish (*Raphanus sativus* L.) and turnip (*Brassica rapa* L.) were sown in naturally infested soil in the field. These species resemble cabbage in the early seedling stage, but they differ in the fact that the storage tissue forms in the hypocotyl and in some varieties in the upper portion of the taproot. Two general types of radish and turnip varieties occur. In those known as the globe and semiround varieties the edible storage tissue consists chiefly of enlarged hypocotyl. In the long or icicle varieties the storage tissue consists of hypocotyl and a certain amount of the upper taproot. In the globe and semiround varieties there are practically no secondary roots on the enlarged storage organ except at the base in proximity to the primary root; in the long varieties the secondary root zones extend from a considerable distance toward the crown of the storage organ.

A comparison of radish and turnip varieties grown in clubroot-infested soil reveals an important relation between the morphology of the storage organs and infection. All radish varieties tested were susceptible. However, in the globe and semiround varieties the clubs were confined to the unenlarged taproots (fig. 4, *B*). In the icicle varieties the lower portion of the storage organ, where secondary roots occurred, was commonly invaded and hypertrophied (fig. 4, *A*). In all radish varieties the hypocotyl tissue remained uninvaded until the latter part of the growing period. If the plants were allowed to remain in the infested soil beyond the usual edible stage, delayed infection of the hypocotyl sometimes occurred and slightly raised blisterlike lesions resulted, but large galls never formed (fig. 4, *C*). However, infection of the hypocotyl was secured readily as in cabbage by wounding the growing tissue in infested soil (fig. 4, *D*).

The same general relation was found in turnip between morphology of the storage organ and infection. In globe varieties the clubs were confined to the regions were secondary roots occur, i.e., the root proper and the base of the storage organ; in the long varieties, the lower portion of the storage organ was commonly invaded (fig. 4, *E* and *F*).

NORMAL ANATOMY OF CABBAGE

In view of the fact that these studies are concerned with the invasion by *Plasmodiophora brassicae* of the lower stem and root of cabbage, the anatomy of these tissues will be described briefly. Germination in cabbage is epigeal. As the hypocotyl expands the cotyledons appear above ground. As pointed out by Woronin (*6*) and Smith and Walker (*5*), the primary tissues of the root possess a diarch protostele with exarch arrangement of the xylem and two alternate

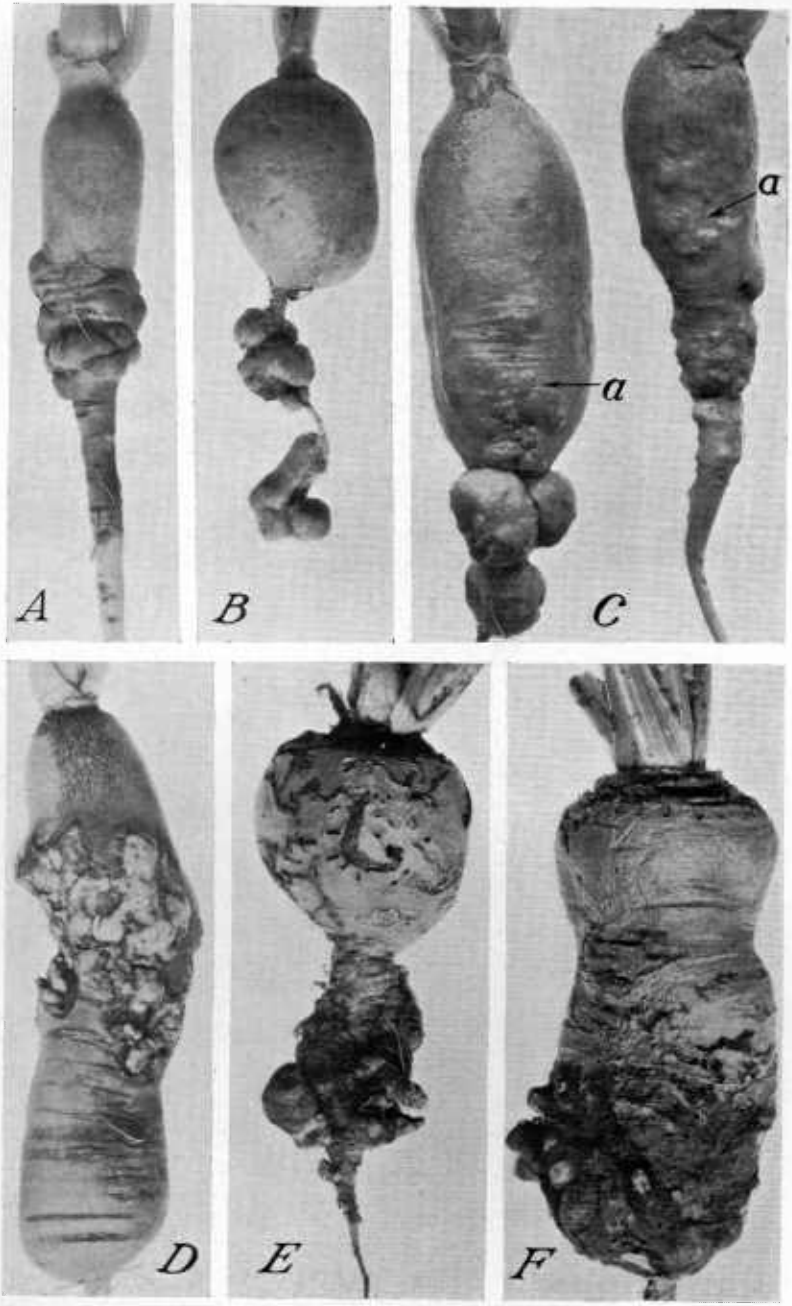

FIGURE 4.—Radish and turnip varieties grown in clubroot-infested soil: *A*, Icicle type of radish in which infection is confined to the enlarged taproot in the region of secondary laterals. *B*, Infection of taproot of globe type of radish, note that the fleshy hypocotyl is free from infection. *C*, Blisterlike areas on fleshy portion of hypocotyl (*a*) resulting from late infection. *D*, China Rose Winter radish; infection following injury to upper portion of fleshy hypocotyl. *E*, Shogoin variety of turnip, globe type; infection is confined to the taproot. *F*, Cow Horn variety of turnip, oblong type; infection is general in the fleshy taproot.

prominent strands of primary phloem. The pericycle is clearly distinguishable just outside the vascular elements. The next outer layer is the endodermis with Casparian strips sometimes discernible. The cells of the inner layer of the cortex possess characteristic thickenings which appear first in the radial walls. Concomitant with the loosening and sloughing of the cortex is the development of the permanent periderm, which arises from the tangential division of pericycle cells. This tissue replaces the primary cortex as a protective tissue, and its development takes place earlier in the root than in the hypocotyl. A cross section of the older root shows most of the tissue to be secondary, consisting of a narrow periderm surrounding the vascular stele. Most of the phloem is secondary and occupies less space than the xylem; intraxylary phloem does not occur.

In the cabbage the hypocotyl is predominantly rootlike in structure; the two exarch strands of the primary xylem of the root continue through the major portion. The first indication of hypocotyl and stem transition is the presence of a pith. The two protoxylem points are gradually separated by the parenchymatous pith cells as they approach the cotyledonary axis, and the pith cells become increasingly abundant in the upper portion of the hypocotyl. Cambium appears early in the differentiation of tissues, and the vascular elements soon appear as a continuous cylinder of secondary xylem and phloem broken by rays.

With the increase of vascular tissue, the cortex is sloughed, but persists longest in the region just below the cotyledonary axis. As in the root, tangential division of the pericycle gives rise to a prominent periderm at the periphery of which cork cambium develops. The change from the exarch condition to the endarch is rather abrupt, beginning in a majority of cases about 1 mm below the cotyledonary node and becoming practically complete at the node.

Cabbage seedlings vary as to the length of the transition region. If the hypocotyl is short the change in type of stele is rather abrupt. The strands of the cotyledonary axis supply the cotyledons, and portions of these strands appear to undergo a transition just below the cotyledonary node, each exarch trace being replaced by two endarch strands. Scattered among the phloem cells in the hypocotyl are elongated, lignified phloem fibers. These sclerenchymatous cells serve as strengthening tissue and are to be found only in the hypocotyledonary region.

The lower stem of the young cabbage plant has a dictyostele type of arrangement. The vascular bundles separated by narrow rays, enclose a conspicuous pith. The pericycle, several cells in thickness, separates the vascular tissue from the cortex. Pericycle fibers differentiate early in the maturation of the lower stem and are more prominent than in the upper stem. Cambial activity in the internodes of the lower stem soon results in a conspicuous cylinder of secondary xylem and phloem tissue. In this region the ray cells become thick-walled and a woody cylinder results. Beginning at about the second node, the medullary rays become gradually broader in the successive internodes, and a more succulent type of stem tissue is apparent. Throughout this region active division and enlargement of the parenchymatous cells of the pith take place. This proliferation, together with a limited cambial activity, is largely responsible

for the increase in diameter of the stem as the head is formed. The epidermis of the stem is somewhat cutinized and waxy.

Cabbage seedlings vary a great deal in length of hypocotyl and stem internodes. Temperature and light have a direct effect on this elongation. At the first node above the cotyledons three separate leaf traces appear and remain as distinct vascular strands in the petiole: these pass to the leaf blade without change of structure. In the region of the node the exit of leaf and branch traces leaves conspicuous gaps or interruptions in the vascular cylinder of the stem. However, they are limited in their longitudinal extent.

In normal cabbage stems, when leaf abscission occurs, a definite layer forms at the base of the petiole. Suberization of the parenchyma cells beneath this layer takes place, and a corky leaf scar is formed in which the vascular bundle scars are prominent.

Further studies of the developmental anatomy of cabbage root, hypocotyl, and stem are desirable.

PATHOLOGICAL ANATOMY OF CABBAGE

Tissue invasion by *Plasmodiophora brassicae* as well as tissue response is best determined by actual observation of the plasmodia in the structures of the host, as seen in serial sections cut both longitudinally and transversely. The relation of the parasite to the cabbage root will be discussed first.

Root Invasion

Most observations of the diseased root of cabbage plants reveal the fact that many of the clubs or overgrowths are not the irregular swellings that one might expect. If a number of diseased cabbage roots are brought together and examined it will be found that, although they may differ greatly in size, most are alike in general outline. The clubs produced on root structures are definite spindle-shaped tumors (fig. 5). When several points not very distant from one another become infected the swellings may fuse in such a way as to give rise to an irregularly shaped compound spindle. In older clubs secondary or adventitious roots arising from the club tissue may become infected and distort the original form of the spindle-shaped club.

In the diseased root of young cabbage, as seen in cross section through the thickest portion of the spindle, the position and the relative size of the primary xylem usually remain normal, since infection occurs after these tissues have differentiated and matured. Malformation is largely confined to the secondary structures, and the primary tissues are invaded only in advanced stages of infection. The most striking deviation from the normal is the presence of few and widely separated fibrovascular bundles. These xylem elements, singly or in small groups, radiate from the primary xylem. The vessels of the secondary xylem are usually smaller than in the healthy root, and are separated by abnormally broad rays, whose cells have greatly increased in size and number. The phloem can sometimes be identified, but with difficulty, by the smaller cells sometimes arranged in rows, as contrasted with the larger irregularly arranged cells of the cortex or periderm. There is no apparent continuous cambial ring as in the normal mature root. A most striking feature of the periderm and of the xylem rays is the cell groups of from four to many cells lying together which are occupied by the plasmodia of the parasite.

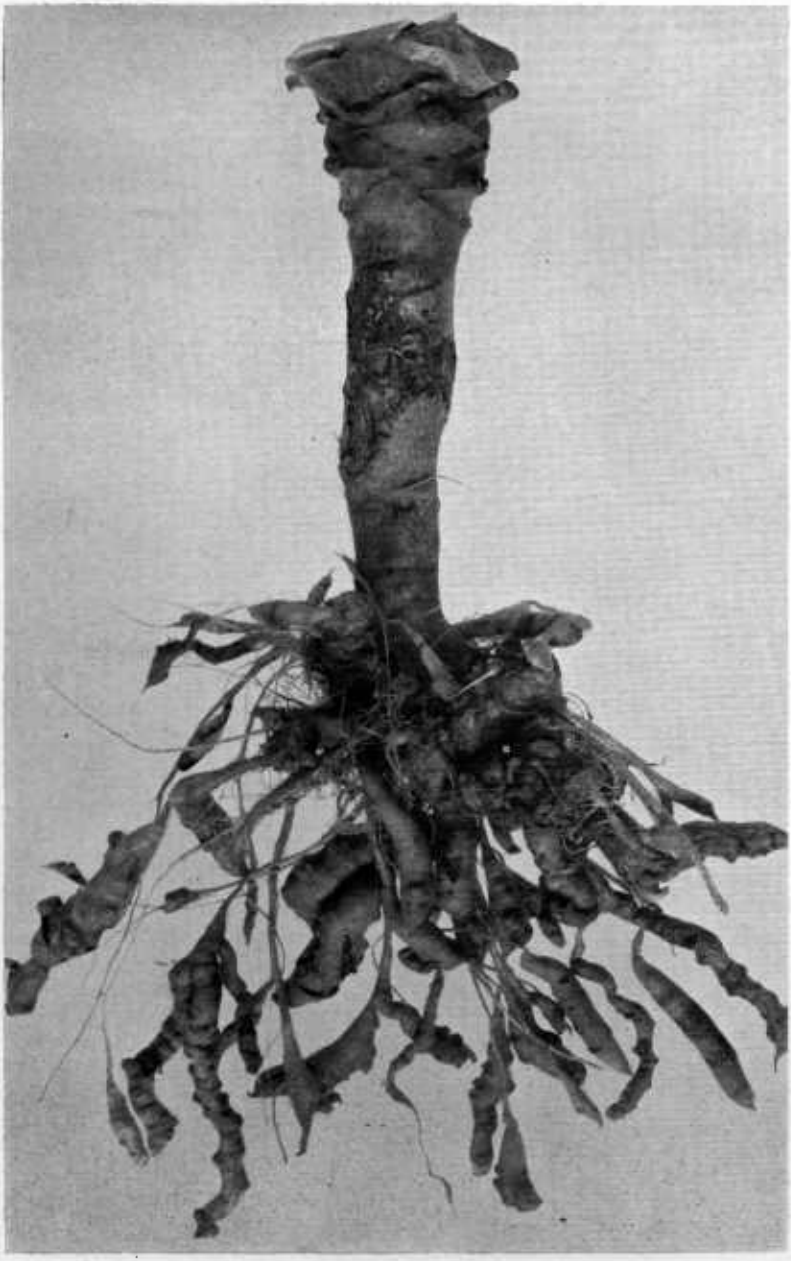

FIGURE 5.—Three-month-old cabbage, showing the characteristic spindle-shaped clubs following infection by *Plasmodiophora brassicae*. Natural field infection.

Hypocotyl Invasion

In the hypocotyl the parasite, upon entrance, migrates through the periderm and phloem rather promptly, going more or less directly to the cambium, as Kunkel (*2*) reported. There is, however, tissue stimulation and cell multiplication in the tissues outside the cambium as the parasite invades the host. It is in the cambium that progress is most rapid, and migration of the young plasmodia in this tissue is directly associated with the development of the cambial cells. Division takes place more freely in the phloem initials than in the xylem initials, and increase in the circumference of the cambial cylinder is brought about by radial and transverse division of these initials. The elements cut off on the phloem side remain undifferentiated and continue to divide and multiply.

Figure 6, *A*, represents a longitudinal section of a cabbage hypocotyl showing periderm infection through a needle puncture. The infected cells are enlarged and more numerous, while those in the uninvaded region remain normal. As a result of the increased cell activity caused by the presence of the parasite, the diseased cells are gradually pushed outwards in the radial direction by cell division beneath. Except for the tissues near the point of original invasion of the parasite, the phloem, pericycle, and periderm become infected only from the diseased cambium. As the young plasmodia infect the cambium tissue either by direct cell-wall penetration or by host-cell division, migration to the sides around the hypocotyl, as well as up and down the cambium, is directly associated with the hyperplasia in this tissue. The greatest growth stimulus is in the region of the infested cells.

Figure 6, *B*, represents a median longitudinal section of the upper hypocotyl, which is the transition region between protostele and siphonostele. The greatest hypertrophy is in the lower portion of this region of transition where protostele type of vascular arrangement occurs. The development of the spindle-shaped club is directly associated with the rapid cambial activity.

Stem Invasion

In the study of tissue invasion by *Plasmodiophora brassicae*, Kunkel (*2*) stated that the path followed by the infecting plasmodia as they passed through the tissues produced a typical spindle-shaped club in the root as well as in stem tissue. He was of the opinion that tissue hypertrophy of root and stem resulted largely from the abnormal growth of the infected cambium, and that most of the cortical tissues became infected from within. This type of tissue invasion and response is typical of the simple protostele, as is found in the root and hypocotyl of cabbage, but is not in accord with the type of response found by the writer in the first and upper nodes. Kunkel's work was, from his description and figures, largely with hypocotyl tissue, which is rootlike rather than stemlike in structure.

He reported the formation of spindle-shaped clubs on cabbage stems artificially inoculated with infectious material. The clubs produced were spindle-shaped, but they were thicker on the side of the stem to which the inoculum was sealed. As has been pointed out, the writer duplicated this type of inoculation on the hypocotyl and secured the spindle-shaped clubs (fig. 3, *E* and *F*). When infectious

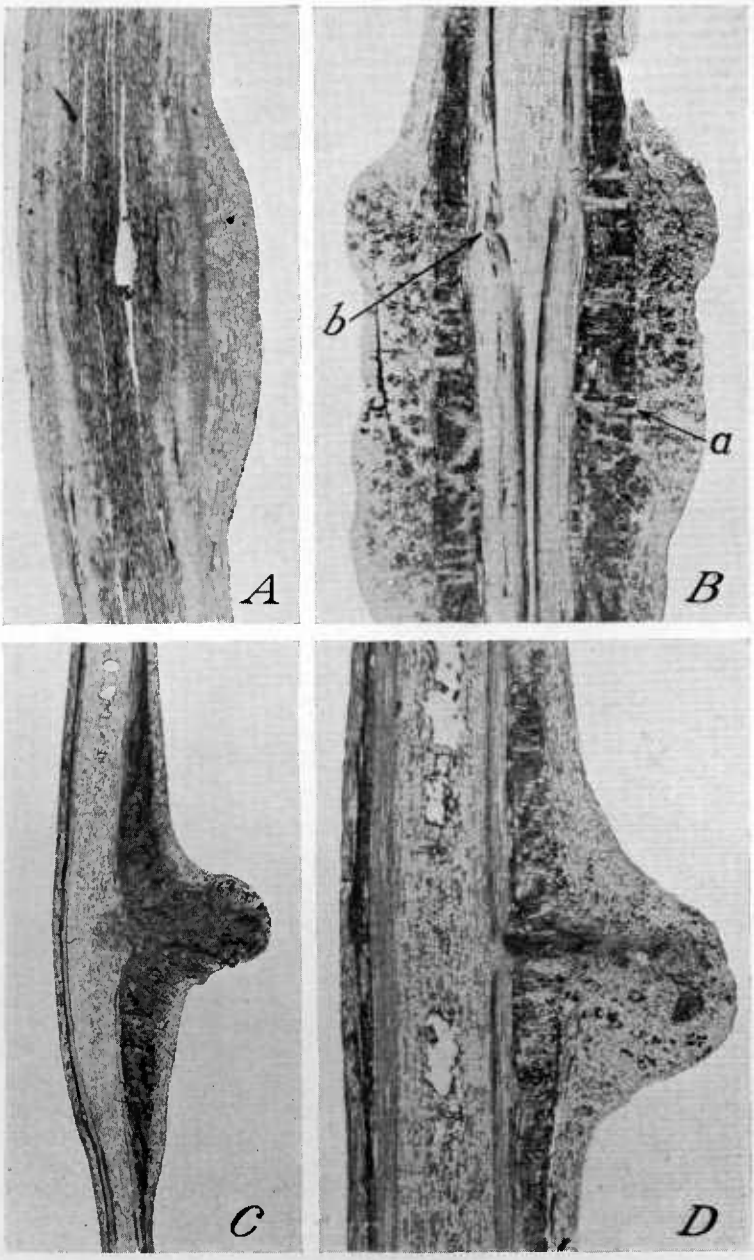

FIGURE 6.—Median longitudinal sections of cabbage plants infected with *Plasmodiophora brassicae*: *A*, Hypocotyl, showing early infection of periderm following penetration through needle wound. *B*, Diseased hypocotyl, showing region of transition between protostele and siphonostele; infection occurred through needle wound at (*a*). The hypertrophy, extending the entire length below the cotyledonary axis (*b*), has resulted largely from cell division in the stele. *C*, Infection from a needle wound in the first internode; the hypertrophy is intermediate between the spindle and the spheroid type. Advanced cortical and cambial infection is evident. *D*, Infection of second stem node, wounded by the removal of leaf petiole; the hypertropby has resulted largely from cortical cell division.

material was applied to the first internode, infection occurred with spindle formation in the direction of the hypocotyl. When this type of inoculum was applied to the upper stem internodes no infection occurred.

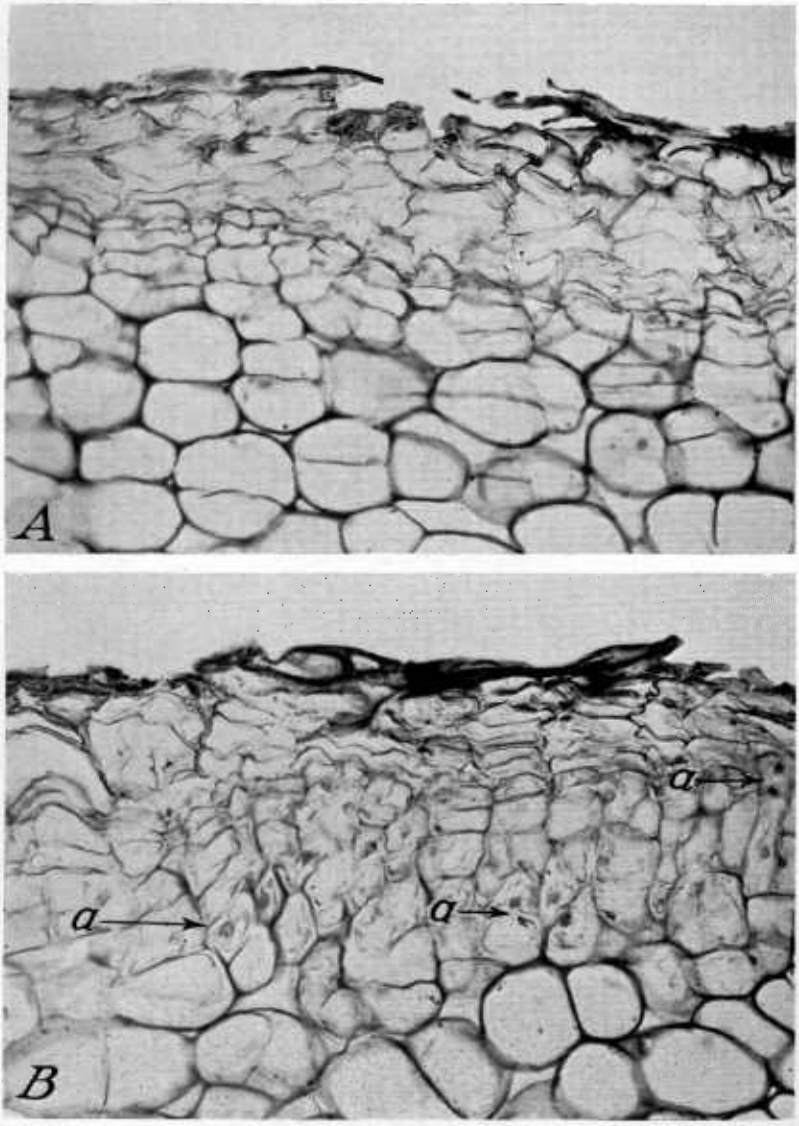

FIGURE 7.—Effect of the application of macerated cabbage root tissue to the first internode of the cabbage stem: *A*, Reaction of the tissue to the application of noninfectious material. The epidermal cells were broken down and cell division in the underlying cortex was stimulated. *B*, Reaction of the tissue to the application of macerated infectious material was similar to that in *A*. Infection has taken place and young plasmodia are below the meristematic layer (*a*). Both ×425.

Histological studies of stained sections of the first internode to which infectious as well as noninfectious material had been applied showed a very definite injury of the epidermal and underlying cells (fig. 7). The cell walls of the epidermis beneath the two types of

inoculum were very much thicker than the normal and stained readily with safranine. Many definite breaks in the epidermal walls were found and an increase in the number of cortical parenchyma cells was evident. This degenerative condition of the host cells no doubt was due to the toxic effects of the infectious and noninfectious root material.

Very early stages of host infection were observed only in tissues beneath infectious material, indicating that host infection took place through wounds resulting from the application of this type of inoculum (fig. 7, *A*). A study of stained sections of unwounded cabbage stem tissue to which diseased soil had been applied as inoculum showed no injury to the epidermal tissue, and there was no evidence of the parasite in the tissues of the host.

In the first internode with siphonostele type of arrangement of the vascular structures the cambial and cortical tissues are more equally active. This results in a hypertrophy which is intermediate between a typical spindle and a typical spheroid gall (fig. 6, *C*).

In the upper stem with a dictyostele arrangement, the plasmodia penetrate the cambium much more slowly than in the protostele (fig. 6, *D*). Migration of the plasmodia of the parasite in the cortical tissues (collenchyma and cortex) causes more proliferation than in the periderm and phloem of the hypocotyl and root. In figure 8 a cross section of a diseased node is represented where a spheroid gall was formed. The infested cortical and secondary phloem cells have divided rapidly, and tissue enlargement in this outer region has contributed very materially to the hypertrophy. The outer tissues have grown until they are many times the thickness of the same tissues in the normal stem. The hypertrophy results more from cell multiplication than from cell enlargement. Although cambial invasion takes place, hyperplasia is chiefly in the cortical tissues. The abnormal multiplication of the host cells is due chiefly to the advance of the parasite. The newly diseased cells formed in the inner tissues force the early activated tissue outward to form the spheroid gall (fig. 1 and fig. 3, *C*).

DISCUSSION AND SUMMARY

This paper presents, for the most part, an accumulation of evidence concerning wound infection and host-tissue reaction of cabbage and other crucifers to the obligate parasite *Plasmodiophora brassicae* Wor. The writer has brought together essential facts necessary to understand the normal anatomy of the plants studied, but no attempt has been made to describe exhaustively the developmental anatomy.

As previously reported by Kunkel (*2*), the spindle-shaped clubs produced on root tissues (protostele type of vascular arrangement) are the distinguishing symptom of the disease. The manner in which the parasite becomes distributed in the tissues of its host as reported by Kunkel (*2*), is characteristic of rootlike protostele structures, but is in no way the reaction observed in the upper stem regions of the cabbage.

In cabbage plants wounded either by the removal of the leaf petioles or by needle punctures, and then grown in diseased soil, club tissue was formed at the points of injury. The clubs that developed in the wounded area of the upper stem were definite spheroid galls, in contrast to the spindle-shaped clubs of the hypocotyl and

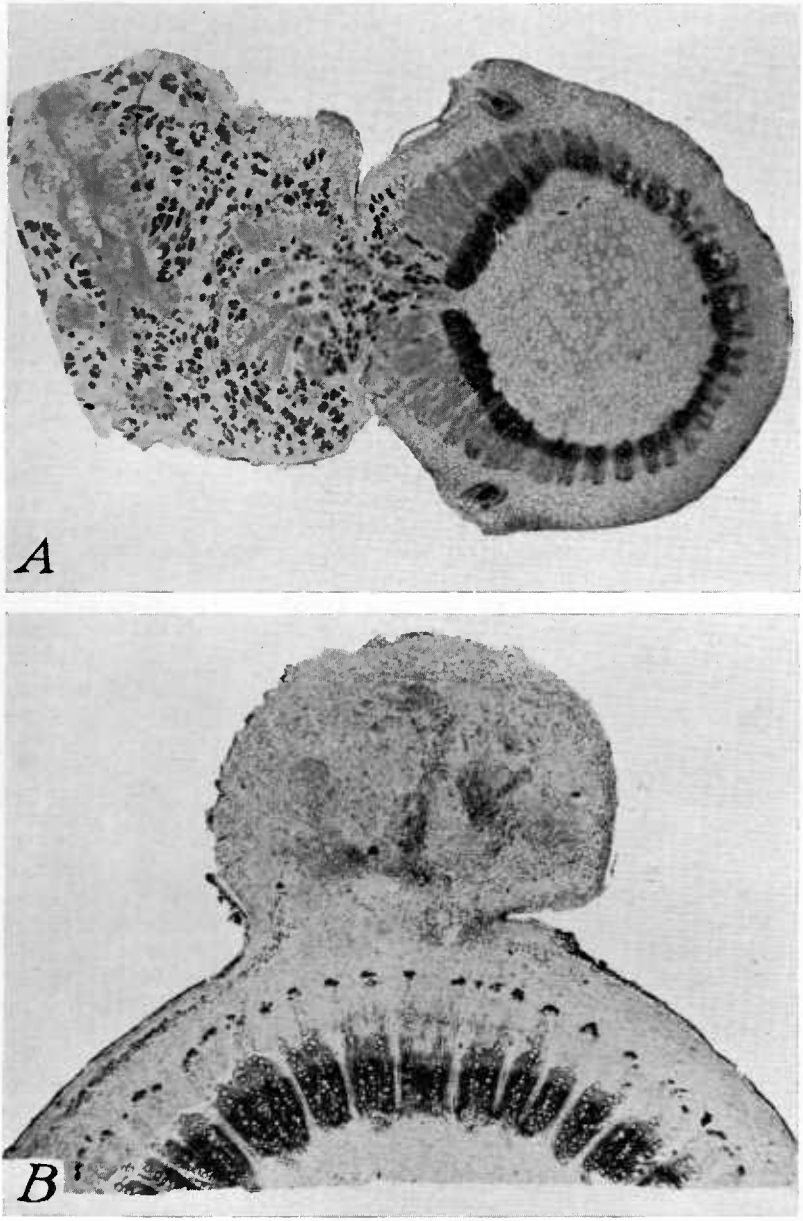

FIGURE 8.—*A*, Cross section of a spheroid gall on a cabbage stem infected through wounded leaf node; a more advanced stage than that shown in figure 6, *D*. Hypertrophy is confined almost entirely to the cortical tissues. The leaf traces can be seen in the cortex at both sides of the gall. *B*, Cross section of first internode, showing very young gall; infection through needle wound; hypertrophy confined to cortical tissues.

root. A somewhat intermediate type was produced on the first internode, a spindle with a gall at its thickest portion. Unwounded plants, as well as those wounded on the stem and allowed to callus before being exposed to the parasite, developed no club tissue on stem or hypocotyl, unless wounds were provided by the formation of adventive roots. When infested soil was applied to uninjured stem or hypocotyl no infection occurred. There was, however, host infection and diseased tissue development when macerated diseased roots were applied to the hypocotyl or the first internode of the stem. Histological studies showed a definite injury to the tissues beneath both the noninfectious and infectious material applied as inoculum and definite cell rupture with breaks or cracks in the host tissue. This evidence, together with earlier observations, indicates quite definitely that infection of hypocotyl and stem takes place through wounds or through ruptures caused by adventive roots.

The formation of a spindle-shaped club, typical of cabbage hypocotyl infection through needle wounds, is largely from abnormal growth of infected cambium, as is true of natural infection of root structures. The plasmodia migrate into the undifferentiated cells on either side of the cambium, but the greatest hypertrophy is in the cambial tissue. Cortical infection and invasion are outward from the infected cambium.

Hypertrophy of the first node (siphonostele) is intermediate between the typical spindle of rootlike structures and spheroid gall of the upper stem.

In the upper stem nodes the hypertrophy is confined largely to the cortical tissues, owing chiefly to the abnormal multiplication of the diseased host cells. The early activated tissue, forced to the outside by newly diseased cells formed as the parasite advances inwardly, results in the formation of a spheroid gall.

A comparison of radish (*Raphanus sativus*) and turnip (*Brassica rapa*) varieties grown in clubroot-infested soil revealed an important relation between the morphology of the fleshy storage organs and infection by the clubroot organism. The globe and semiround varieties of radish became infected only on the unenlarged taproots, where, as in the icicle varieties, the lower portion of the storage organ in the area of secondary roots was commonly invaded and hypertrophied. Infection of the hypocotyl of the radish was secured readily, as in cabbage, by wounding the growing tissue in clubroot-infested soil. All radish varieties tested were susceptible.

The same general relation was found in the turnip between the morphology of the fleshy storage organ and infection.

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