ROOT ROT OF PEAS IN THE UNITED STATES CAUSED BY APHANOMYCES EUTEICHES (N. SP.) [1, 2]

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INTRODUCTION

During the past five years, in which the senior writer has endeavored to determine the causes which produce the root rot of peas known widely in the United States, several fungous diseases have been distinguished which severally or together appear to be responsible for all of the important injury which has been found incident to the underground portion of this plant. One of the less important of these diseases caused by a species of Fusarium has been described in a previous paper (*8*).[3] The most important of these diseases, more important, in fact, than the others combined, is the subject of the present paper. The account of the morphology and taxonomy of the fungus causing the disease and the drawings are contributed by the junior author. Several fungous diseases of pea roots of much local importance have also been studied. Among these are two caused by species of Pythium, one of which in some seasons is almost always associated with Aphanomyces, an association which misled the senior writer in a previous note (*6*) to ascribe to the Pythium alone the injury due to both organisms. The diseases caused by species of Pythium remain to be described in a following paper.

THE DISEASE

DESCRIPTION

The injury caused by a fungous parasite which invades only the subterranean portion of the plant must, of course, be sought in its early stages in the roots themselves. In later stages the top of the plant may be modified in response to the root injury in a way which may be specifically characteristic. In the case of this disease the injury eventually presented by the top of the plant is not characteristic, the form which it takes depending largely upon the stage of development at which the roots became thoroughly invaded and to a lesser extent upon the degree of resistance of the variety of peas. If the plant becomes invaded in the basal stem below ground as well as in the roots before the plant has developed more than three or four nodes, sudden wilting may result. Usually under field conditions invasion takes place later than this, and the result is a general retardation of growth, the death of the lower leaves progressively upward, and finally, when the plant is in full bloom, it may shrivel up completely. More frequently, however, the plant persists in a weakened condition until it has brought its poorly filled pods to maturity.

If extensive infestation of the roots is delayed until the blossoming period, the plants may mature under favorable conditions without any conspicuous indication of injury. These several symptoms have no common factor which distinguishes plants infected with Aphanomyces from those attacked by several other fungi. However, there is one test which can be applied that will often give a decisive indication of this disease. If some of the infested plants are pulled, the stems of those which are thoroughly invaded will fail to break at the seed, as is the usual rule with healthy plants, but the vascular core of the taproot will pull out as a long string

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[2] Results of work done in cooperation with the Agricultural Experiment Station of the University of Wisconsin.
[3] Reference is made by number (italic) to "Literature cited," p. 325.

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with a few of the larger lateral branches. No other type of root rot has been found which permits the pulling out of these vascular strings. This test does not apply in the earlier stages of disease and sometimes fails to give results when the soil is exceedingly hard and dry. Examination of the roots themselves will reveal the extent of injury by the soft decay of the cortex. In early stages there is a pale yellow discoloration of the decayed tissue not readily distinguishable from the discoloration associated with the invasion of the mycorrhizal fungus described in a previous paper (*9*).

The soft decay and shrinking of the cortex of the roots and of the portion of the stem below ground usually distinguishes this disease from the turgid roots with the mycorrhizal fungus. After a time the dead cortical tissue usually becomes blackened (pl. 1) so that it can not readily be distinguished by this character from other forms of root decay. If the visible symptoms already described fail to distinguish the disease with certainty, it is almost always possible at any stage in the development of the disease to distinguish it by an examination of the roots with a microscope. The characteristic spores of the fungus, described later, are always formed more or less abundantly in some of the dead cortex of root (pl. 2) or more rarely in that of the stem, and serve to determine the presence of this parasite beyond possible doubt.

HISTORY OF THE DISEASE

Since this disease has not been distinguished previously under any name, it has no unmistakable written history. There are a few references to pea-root troubles, ascribed to other fungi, but on such insufficient evidence that it is not impossible that they may be pertinent here. The first of these is by Wittmack [4] (*19*), who found in decaying roots of peas sent to him by Sadebeck from Hamburg, Germany, oospores apparently belonging to a new Pythium, which he called *P. sadebeckianum*.

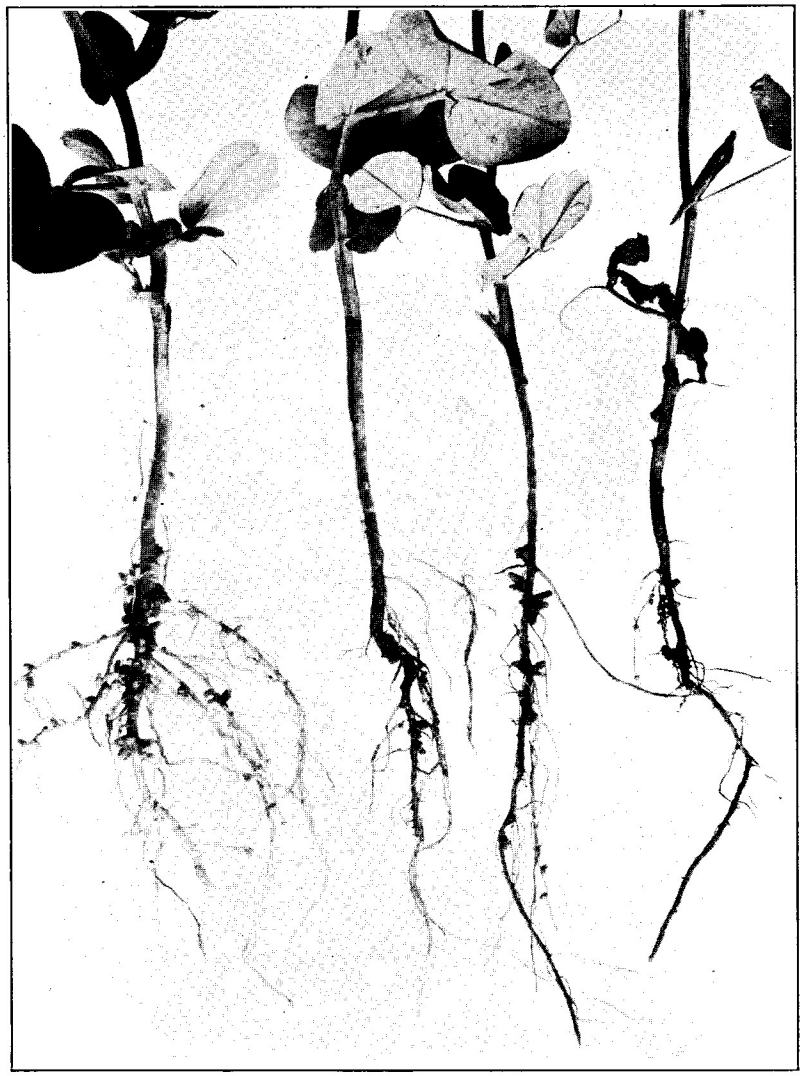
In the United States the disease has undoubtedly been present for a long time and has compelled the abandonment of intensive culture of peas for canning purposes in certain restricted areas. Recently Clinton (*2*) has described a root rot of peas in Connecticut evidenced by the presence of oospores which he believed to be those of *Phytophthora cactorum*. The senior writer has been unable to produce any extensive decay in pea roots growing in soil with a culture of this species, and in no case were oospores formed in the few small lesions produced. Although infection of root ends with a pure culture of a species of Pythium has been obtained by the writer with the formation of oospores in the decaying tissue, and though spores apparently of this species have been found occasionally in field material, nevertheless the measurements and drawings given by Clinton appear for the most part to agree more closely with the spores of the Aphanomyces species, to which reference was made in a brief abstract (*7*) and which is fully described in this paper, than with those of any other fungus that has been encountered. There are other references to rootrot of peas in the United States which undoubtedly indicate this disease, but which do not contain a sufficiently adequate description to make exact determination possible.

THE FUNGUS

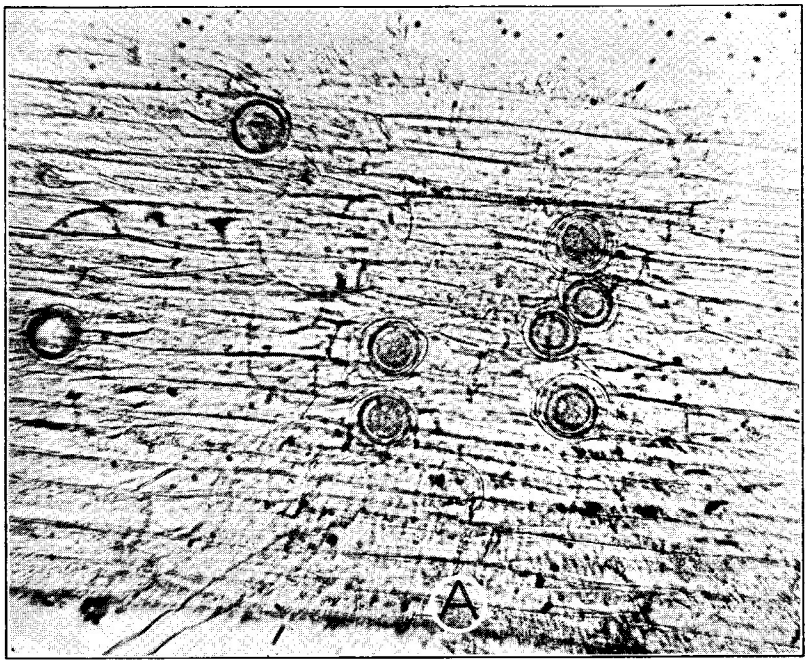
HOST PLANTS

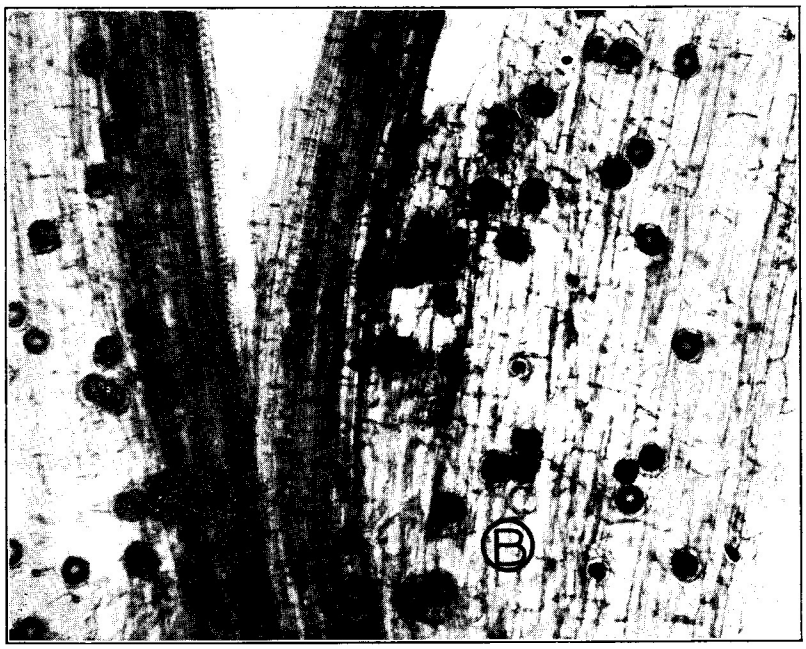
Although it seems unlikely that this fungus is parasitic only on peas, a search for other host plants has thus far been in vain. The roots of many species of plants growing among diseased peas have been examined for the presence of the characteristic oospores and decayed cortex which might have been produced by this fungus. In order to test thoroughly the possible relation of this fungus to the species of Aphanomyces on sugar beets, described by Peters (*13*, *14*), seedlings of this plant and of cress (*Lepidium sativum*) have been repeatedly inoculated without any infection resulting. The conclusion that these fungi are distinct, at least in their physiological capacities, is further borne out by the fact that examination made of fields of beets grown on pea-sick soil, made both by the senior writer and R. E. Vaughan, has discovered no trace of the European disease. On the other hand, although it appears generally true that beets grow well on old pea fields and vice versa, experience is reported otherwise in the Salt Lake Valley, Utah. It may be of interest to note in this connection that in the course of making isolation from diseased peas from this valley with the

[4] WITTMACK, L. UEBER EINE DURCH PYTHIUM VERANLASSTE KRANKHEIT DER ERBSENWURZELN. Paper presented at 64. Versamml. zu Halle, Gesell. Deut. Naturf. u. Aerzte, Sep. 1891. Not published. Title in Verhandlungen 2: 108. 1892.



Root rot of peas, variety Early Market, caused by *Aphanomyces euteiches* occurring in a field at Hanover, Md., May 21, 1920. The plant at the left with white clean stem and turgid roots is not infested. The three remaining plants show increasing degrees of disease progressively toward the right. The plant at the extreme right shows the first symptoms of disease above ground in the shriveled lower leaf





Oospores of *Aphanomyces euteiches* in cortex of the roots of peas inoculated with a pure culture of the fungus

A.—Oospores in a razor section. × about 300
B.—Oospores as they appear in a decaying rootlet crushed under a cover glass. × about 150

writer, John W. Carlson, of the Utah Experiment Station, obtained among other fungi a culture of *Pythium (Rheosporangium) aphanadermatum* (Edson) Fitzpatrick, one of the well-known sugar-beet parasites. This is one of the species of Pythium which the writer has found capable of producing root rot of peas, and if it is widely distributed in this valley it may be the common parasite of these plants which renders pea growing on old beet fields unprofitable.

GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

In the United States the disease has been found in practically every pea-growing district that has been searched with care. In the Eastern and Central States it occurs frequently, and often very destructively. It has been found in Utah, Idaho, and Montana, where it appears to be unimportant at present except under special conditions described later. In the Pacific Coast States it has been found but once, in diseased pea plants sent from Santa Clara, Calif. It appears that, although the disease is very widely distributed, it requires special soil and climatic conditions generally present only in the Eastern and Central States in order to become important when peas are grown intensively. It is probably this disease more than any other factor which has compelled the growing of peas in a comparatively long rotation, and has thus limited the culture of this food crop. Certain it is that were it not for the accumulation of this disease and others with intensive pea culture, the cost of producing canned green peas and probably of dried peas would be greatly reduced. In the absence of any accurate survey of fields in the region where this disease occurs, it is impossible to state approximately the number of acres that are damaged or destroyed each year; in some of the older districts in unfavorable years as much as 25 per cent of the acreage seems to be infested. From the reports of growers and county agents, combined with a limited personal survey, it appears that several thousand acres of peas are rendered unprofitable or destroyed in the United States each year.

ISOLATION OF THE FUNGUS

The fact that this important parasite of peas has remained undescribed so long is undoubtedly due to the difficulties encountered in isolating it in pure culture. One cause of failure is due to the brief period of time during which the fungus flourishes in an active vegetative stage at any point in the host tissue. When the fungus first begins to invade tissue, it can hardly be induced to grow out on the culture medium in preference to the living cells. Only a few days are required at ordinary temperatures to exhaust and destroy the host cells, whereupon the mycelium begins to transfer its contents into the large oospores, which are formed abundantly. After this process is well under way it is again almost impossible to secure growth on culture media. Thus it is necessary to obtain diseased plants in which the fungus is growing vigorously just prior to extensive oospore formation if success in isolation is to be attained.

Another cause of failure is due to the large number of vigorous saprophytes which follow closely the invading Aphanomyces. Besides abundant bacteria, in some seasons there is almost always present a species of Pythium so much more vigorous in growth upon culture media that it usually submerges the Aphanomyces. As yet no method of surface disinfection tried has given material aid in destroying the saprophytes present. The parasite is extremely sensitive to bichloride of mercury, and its use almost invariably brings failure.

In order to secure cultures of the fungus from localities remote from facilities for making isolations or from plants which are so far decayed that direct isolation is impossible, the senior writer has been accustomed to pack diseased roots with soil from the field in tin cans kept tightly sealed awaiting a convenient time in which to combine this material with steam-sterilized soil. In this soil mixture peas are then grown properly protected from outside infection until plants are obtained which have developed the stage of the disease suitable for the isolation of the parasite. In this way cultures have been obtained for comparison from nearly all of the regions where the disease has been found. Fragments of tissue are selected in which the mycelium of the fungus is seen under the microscope to be filled with granular contents, are thoroughly washed in sterile water, and are placed on plates of 2 or 3 per cent clear agar, or preferably prune agar, as recommended by Hartley (4) for the isolation of Pythium. The parasite always grows sparsely, sending out long, straight filaments with comparatively short lateral branches through or over the agar. It will almost always outgrow the bacteria which soon develop abund-

antly. Fragments of agar containing ends of these strands are cut from the plate and transferred to new plates until no bacterial growth accompanies the fungus, when it is transferred to corn-meal agar for a stock culture. It often happens that strands of Pythium grow in a manner resembling Aphanomyces so closely that they can not be distinguished until they are transferred to new plates of prune agar, where they soon develop a white matted growth very different in character from the sparse arachnoid growth of Aphanomyces. Oftentimes many fragments of roots and stems must be selected and plated before a culture of the desired fungus is obtained.

MORPHOLOGY OF PARASITE

MYCELIUM

In longitudinal sections of diseased pea stems the vegetative stage of the parasite is revealed as a hyaline nonseptate mycelium, composed of hyphae, varying considerably in diameter among themselves, but individually not subject to abrupt fluctuations in respect to this dimension. (Pl. 3, A.) Branching is exhibited in moderate, usually not in great abundance, the branches generally being produced at angles approaching a right angle. Not infrequently branches show little linear growth, then remaining as short diverticulate spurs on the axial filaments. The fungus is largely intracellular, the hyphae being oriented longitudinally within the cells, their development between the cells being relatively meager and apparently more or less accidental. Appearances often suggest that the fungus passes through the cell walls of the host perhaps with less ease than, for example, some parasitic species of Pythium. Whereas in cortical tissue invaded by the common damping-off fungus, the hyphae pass promiscuously from cell to cell, without much evidence of the membranes providing any obstacle, in tissue invaded by the root rot parasite cells crowded with mycelium may lie adjacent to others entirely free of the fungus. It should be mentioned, however, that the cortical tissue invaded by the root rot parasite is of a distinctly less succulent character than the cortical tissue of seedlings of various hosts subject to damping-off, and that strains of Pythium effective in producing the latter type of injury exhibit little or no aggressiveness toward pea plants readily attacked by the species of Aphanomyces under consideration. A somewhat similar mycelial distribution was reported by Weatherwax (*17*) in filaments of *Spirogyra dubia* Kg. invaded by *Aphanomyces phycophilus* DeBary.

SEXUAL REPRODUCTION

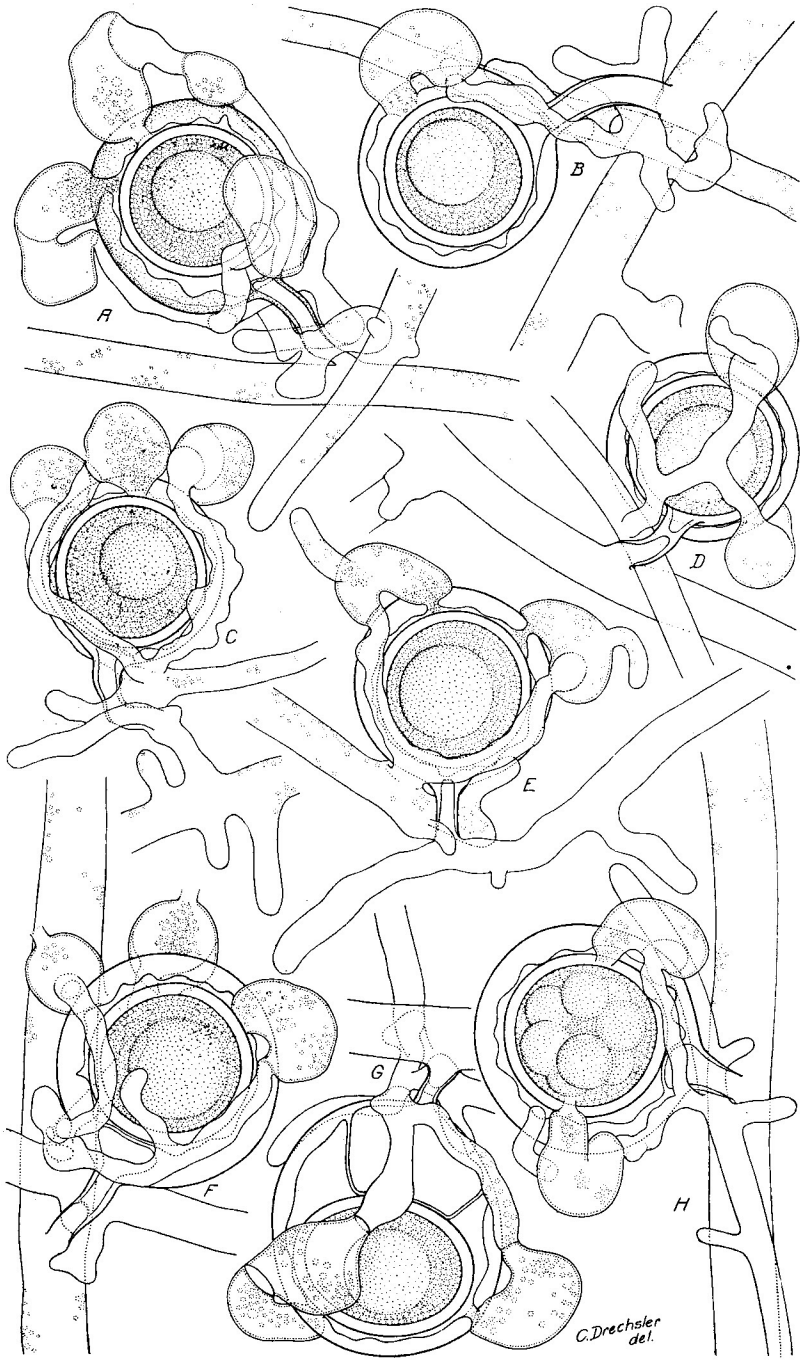
The purely vegetative condition represents a rather brief phase in the development of the fungus, mycelial growth coming to a pause as the cortical tissue begins to collapse. Oogonia and antheridia now make their appearance. Owing to the crowding of the hyphae within the host cells, the relation between the branches bearing these organs can not usually be distinguished. There is no reason to believe, however, that the sexual apparatus in diseased plants shows any significant departure from these structures as they develop on suitable artificial substrata where they can be accurately studied. The oogonia before fertilization are thin-walled, subglobose bodies with densely granular, vacuolate contents. After fertilization the oogonial wall becomes conspicuously thickened, the thickening being subject to peculiar irregularities, with the result that the inner contour is represented by a more or less sinuous line, giving the whole structure a peculiar internally scalloped appearance. (Pl. 4, A to H.) As in other species of Aphanomyces, the oogonial cavity is very largely but not completely occupied by the single oospore, a subspherical structure with a thick colorless wall, the thickness of the latter not given to great variations either with respect to different individuals or with respect to different portions of the same individual. The contents of the normal mature oospore consists of a

EXPLANATORY LEGEND FOR PLATE 3

A.—Portion of longitudinal section of basal part of pea stem in an early stage of infection, showing the development of the fungus in certain cortical cells and extension into adjacent cells. The ellipsoidal body near top of figure represents a developing oogonium. From material of a plant grown in soil inoculated with a pure culture of the parasite, killed in Flemming's weaker solution, embedded in paraffin, cut on the microtome, and stained with Flemming's triple combination. ×470

B.—Epidermis of hypocotyl of pea seedling derived from a surface-sterilized seed planted on sterile water agar in a test tube, the resulting culture inoculated 5 days after planting with a pure culture of *Aphanomyces euteiches*. Showing penetration by 3 extramatrical hyphae into tissue of plant—one, *Ba*, entering close to or at the juncture of adjacent epidermal cells; another, *Bb*, entering between two epidermal cells; and a third, *Bc*, entering between a guard cell and an epidermal cell. ×470

(For explanatory legend see p. 298)



Various sexual apparatus of Aphanomyces euteiches from 20 day old hard corn-meal agar cultures, illustrating close approximation of antheridial and oogonial stalks; branching of antheridial stalk; size, shape, and interrelation of oogonia and antheridia; character of cross walls setting of sex organs; and occurrence of diverticulate branches. Drawn with the aid of a camera lucida at a magnification of 1,580 diameters, and reduced in reproduction to a uniform magnification of 470 diameters

large, spherical, somewhat eccentric, vacuole-like body of apparently homogeneous structure, surrounded by a matrix containing numerous small uniform granules in regular concentric arrangement. The literature on the genus Aphanomyces like that on the Saprolegniaceae in general, shows some difference of opinion with reference to the nature of these internal elements, the large central body sometimes being referred to as an oil globule (*1*, *3*, p. 160); while evidently at other times (*3*, p. 10) it is regarded as being of a protoplasmic nature and the granule-like structures as consisting of oily matter. Since the small peripheral granules stain well with Sudan III in the species under consideration, it would seem the latter view is probably more nearly correct, although the apparent homogeneity of the material constituting the central globule can not be regarded as characteristic generally of protoplasm.

The relationships of the sexual apparatus can be studied to excellent advantage when the fungus is grown on hard corn-meal agar. By cutting off a thin surface layer of the substratum, a thin preparation is obtained containing the thallus at nearly a uniform optical level without any serious disruption of the structures concerned. Typically, and at least in the large majority of cases, the male and female organs arise from hyphae which show no close organic connection. The oogonium is apparently always terminal, being generally borne on a rather short stalk arising as a lateral branch from a hypha usually of more than ordinary diameter. The antheridial branch generally arises from a less stout filament, which in many instances will be found to cross the larger filament close to the points from which the sexual branches have their origin. (Pl. 4, A to H.) Usually the antheridial stalk becomes intimately involved with the oogonial stalk after making a partial turn about the latter, although the condition described by Von Minden (*11*) for his *Aphanomyces helicoides*, and figured by Kasanowsky (*10*) in his account of *A. laevis*, in which the antheridial stalk makes several distinct turns about the oogonial stalk, has never been found realized. A number of short diverticulate branches often are borne on the hypha from which the antheridial branch originates (pl. 4, A, B, E, H) and occasionally on the hypha to which the oogonial stalk is attached. (Pl. 4, C, E.) As far as can be determined, they serve no special purpose, but add to the characteristic involved appearance of the apparatus, thereby further enhancing the optical difficulty in resolving it with certainty.

The antheridial branch may be simple or branched, the branching frequently occurring near the base of the oogonium or a short distance from the base. Usually it is limited to a single bifurcation (pl. 4, B, H), but instances in which one (pl. 4, A) or both (pl. 4, F) of the resulting elements branched again have been found, one of the ultimate elements then sometimes failing to develop any male organ. (Pl. 4, D, F, G.) The antheridium is always an expanded structure set off from the stalk by a septum, which is frequently curved with the convexity protruding into the interior. Often the antheridium appears sharply arched in the manner of a measuring worm, the fertilization tube in such instances usually being produced in the region where the distal lobe is in contact with the oogonium. (Pl. 4, B, H.) Not rarely a hyphal diverticulum is present as a dorsal appendage (pl. 4, E, F) quite similar in appearance to analogous protuberances that can be observed occurring singly on the male organs of some species of Pythium and, as in the latter forms, apparently not serving any evident purpose.

In some instances when the antheridium attains unusually large proportions a transverse septum may be inserted, generally at a constriction. (Pl. 4, A, G.) The resulting structure may evidently be regarded either as two male organs developed in series on the same stalk or as a compound antheridium. Sometimes both of the male elements thus delimited have been observed communicating with the interior of the mature oogonium by independent tubes or apertures in the oogonial wall. While the origin of the antheridial branch near the base of the oogonial branch and a sort of contact relation of the two are of common occurrence and characteristic of the fungus, male stalks altogether unrelated to the oogonial stalk in place of origin also occur. As the number of antheridia to an individual oogonium varies from one to four, or even five, considerable variety in origin may be expressed in a single sexual apparatus.

The oogonia and oospores developed in culture are quite similar to those found in diseased host tissue, but a few details not easily observed in the latter substratum may here be studied to advantage. Thus the peculiar thickening of the oogonial wall will be seen produced into the distal portion of the supporting stalk, diminishing rather markedly, so that the latter is repre-

sented in its proximal portion in an entirely unmodified form. An interesting variability is exhibited by the partition delimiting the oogonial cavity at the base. Usually it occurs as a rather inconspicuous cross wall inserted in the distal portion of the stalk, providing no marked interruption in the roughly subspherical interior surface of the oogonial envelope. (Pl. 4, A, F, H.) It sometimes projects into the interior as a convex columella-like structure, and in some cases such development may be so pronounced that the available space is greatly reduced, constraining the oospore to adopt a distinctly ellipsoidal shape, with the major axis transverse to the axis of the oogonium. (Pl. 4, G.) Such modification may perhaps best be regarded as a fortuitous morphological peculiarity rather than as an abnormality, being present in material derived from the diseased host as well as in culture and evidently not adversely affecting the vitality of the oospore.

The processes of fertilization are not as amenable to direct observation as might be desired, for although an abundance of antheridia and oogonia develop readily in liquid culture, and consequently can be obtained in Van Tieghem preparations, their contents appear to degenerate at an early stage, or an oospore will be produced of a patently abnormal character, exhibiting a promiscuous granular or irregularly vacuolate internal structure. Examination of preparations of corn-meal-agar cultures abounding in normal sexual conditions indicate that when several antheridia are present, as is usually the case, all or several of them may develop fertilization tubes. For example, out of three or four antheridia attached to an individual female cell, two or three may usually be found to communicate with the interior of the oogonium by openings through the thick oogonial wall. While not all of the antheridia provided with such communications appear devoid of contents, it is certainly not unusual to find two male organs from which the protoplasm has disappeared completely or almost completely. (Pl. 4, A, F, H.) It is readily apparent that such a condition might be brought about by degeneration quite as well as by evacuation of contents into the oogonium. In a cytological study of a congeneric species, Kasanowsky (*10*) found that after fertilization was effected by one antheridium the nucleus of a second antheridium was intercepted in its passage through the fertilization tube. No statement is made by this author whether or not any transfer of cytoplasmic material may take place from a second antheridium previous to the entrance of the nucleus into the tube and its interception. The empty condition of plural antheridia, frequently encountered, points to such possibility. It is even not inconceivable that where nuclear degeneration is as easily effected as in the sexual apparatus of the coenocytic type, to which Aphanomyces seems to belong, the entrance of a supernumerary nucleus may not bring about as impossible a cytological situation as sometimes has been assumed.

GERMINATION OF OOSPORES

The germination of oospores of Aphanomyces appears to have been recorded for only two species, DeBary (*1*) having observed the process in material of *Aphanomyces stellatus* De Bary that had been kept in water for three months, and Kasanowsky (*10*) in material of a form he designated as *A. laevis*, DeBary, after this had passed through a resting period of over seven months. In both cases a germ tube was produced which perforated the oogonial wall and developed into a mycelium. A difference in the accounts of these authors may be noted in the promptness with which branching of the germ tube occurred, that of *A. stellatus* giving rise to a large number of hyphae very soon after emerging, if not immediately, while in Kasanowsky's fungus ramification was delayed until the tube had attained a length of 300 μ.

Unlike oospores of the two species mentioned, those of the parasite attacking peas require no extended resting period. When material from 15-day-old corn-meal-agar cultures was transferred to hanging drops in Van Tieghem cells, a considerable proportion of the oospores germinated, the method of germination, whether by mycelial development as observed by DeBary and by Kasanowsky, or by the production of zoospores which has not hitherto been recorded for any member of the genus Aphanomyces, seemingly being dependent to a great extent on the amount of nutrient material incorporated in the preparation. Thus Van Tieghem cultures prepared from material without any preliminary washing or removal of particles of substratum quite invariably exhibited direct germination into a mycelium. When the material was first allowed to soak, and the bits of solid food material removed as completely as possible, indirect germination by means of zoospores predominated. The initial changes which take place during the first 24 hours appear to be the same, regardless of eventual developments. The

thick oospore wall disappears as such, being reduced to a delicate membrane, which yields to the enlargement of the protoplast and is pressed against the inner surface of the oogonial wall, with the result that the appearance is presented of the oogonial cavity being entirely filled with protoplasmic contents. These contents, moreover, no longer exhibit the geometrical arrangement of the resting condition, but show instead a more or less uniformly granular condition.

If the conditions are such as to favor direct germination, one or several protuberances are now thrust through the oogonial wall. (Pl. 5, G, H.) The communications established by the fertilization tubes seem to serve an important rôle as channels of egress, as the granular germ processes can frequently be clearly seen passing through them into the antheridial cavity and thence emerging by a perforation through the antheridial wall; and owing to the collapse of the empty antheridia and difficulties in observation, instances of such utilization of these aperatures are very probably even more numerous than could be definitely established. In many cases the germ tube immediately gives rise to a number of branches, usually aggregating about a dozen, which thus occur in rather crowded, bristling arrangement not at all typical of mycelial ramification in the species (Pl. 5, L, K); in other cases branching is delayed until the germ tube has attained considerable length, and the sort of ramification then exhibited is altogether comparable to that shown by the thallus of the fungus generally. (Pl. 5, I, J.) Manifestly the conditions here represented correspond to those found in the two organisms investigated by DeBary and Kasanowsky, respectively. In appearance the resultant structures bear some similarity to those produced by the two types of direct germination of, for example, the sporangia of certain species of Phytophthora. They may, indeed, plausibly be explained in the same way—vegetative development of the oospore as a single energid, on the one hand, as contrasted with the development of multiple energids resulting from division processes incident to abortive zoospore production, on the other.

When an oospore germinates by the production of zoospores, a single filament is produced which ceases elongation after attaining a length varying from 8 to 12 times the diameter of the oogonium. (Pl. 5, E.) The germ hypha regularly decreases in width toward the tip, the distal portion usually measuring about 4 μ in this dimension, or somewhat less than one-half the diameter of the basal portion. At this stage, when only approximately half of the oogonial contents have passed into the germ hypha, zoospore formation is initiated. In the germ hypha the process shows no departure from the usual course that has been described so frequently in the filamentous vegetative sporangia of other members of the genus, yielding from 6 to 10 cylindrical portions of protoplasm connected, at least for a time, by a greatly attenuated strand. (Pl. 5, A.) It appears probable that development is in the main acropetal, the divisions delimiting the two or three most distal portions having been observed to be initiated after the separation of the basal portions had been effected, the last division of all setting off the terminal portion. Within the oogonial wall the residual material which has become concentrated in a subspherical mass near the orifice of the germ hypha undergoes similar cleavage, as evidenced by its segregation into lumps that become increasingly distinct and after some mildly writhing movements assume individuality as independent subspherical protoplasmic masses. (Pl. 5, A.) Suddenly the tip of the germ hypha gives way and the protoplasmic masses escape one by one in rapid succession, each rounding up and encysting near the orifice. An interesting feature in the evacuation of the germ sporangium is that the globose bodies within the oogonium enter the germ hypha at the base to replace distal ones by streaming through the small aperture in the oogonial wall and assuming the cylindrical form of the filament. In the course of about 10 seconds the entire apparatus is emptied. (Pl. 5, B.) The discharged spores are scattered loosely about near the mouth of the germ hypha or collected in whole or in part in a loose irregular aggregation. (Pl. 5, C, D, and F.) They number generally from 13 to 18 (most frequently 15), depending apparently somewhat on the size of the oospore and the proportion of oversized individuals capable of producing one compound or two normal motile forms.

While sporangial germination of the oospore does not appear to have been recorded hitherto for any species of Aphanomyces, it may be mentioned that a somewhat analogous development was noted by Sorokine (*16*) in the germination of globose bodies belonging to *A. stellatus*, which he designated as conidia. An extensive

the thick wall of these bodies through which the germ tube made its exit and their considerably inferior length provide differences in detail. Direct germination by a germ tube was also described for these conidia, the resulting hyphae being of the more remotely ramifying type, generally characteristic of the growing mycelium.

ASEXUAL REPRODUCTION

It is presumably altogether safe to take for granted that the sexual spores developed in the tissues of the diseased host constitute the regular resting bodies of the fungus, by the germination of which the parasite is reestablished in successive seasons. That by the production of germ sporangia they are also the chief means by which the fungus extends its distribution in the soil, appears at least very probable, although, as is well known, dissemination of the aquatic members of the genus is effected by zoospores produced in sporangia of mycelial origin. For when infected pea tissue containing an abundance of the mycelium of the parasite is placed in water no extramatrical development takes place, the organism thus differing considerably in behavior from the amphibious species of Pythium or Phytophthora, for example, which are frequently found in similar relationship, as well as from the congeneric form reported by Peters (*13*, *14*) as causing root rot of sugar beets in Germany. The pea parasite, however, continues in its development of sexual spores apparently uninterrupted. But even if zoospores could be produced by such means it is not certain, in view of the brief time elapsing between full mycelial development and the initiation of sexual stages, that extensive zoospore formation from mycelial elements could frequently be expected in nature.

On the other hand, in artificial culture, the production of zoospores from the ordinary filamentous sporangia characteristic of the genus can be induced, and that in exceedingly great profusion. Following the well-known methods for cultivating aquatic forms, the parasite was grown in pea decoction made by adding from 8 to 10 freshly shelled peas to 100 cc. distilled water in an Erlenmeyer flask and sterilizing by autoclaving or intermittent steaming. Altogether satisfactory results were also obtained by the use of canned peas with some of the liquor in which they were obtained, as well as by employing about twice the number of dried peas to the same quantity of water. Within three or four days at ordinary temperatures an extensive submerged mycelium was produced, appearing in the liquid medium as a translucent nebulous mat. The whole growth was now transferred to a deep Petri dish, the peas removed, and the mycelium washed several times at intervals of about 15 minutes with changes of sterile water. For convenience in examination it was found desirable the last time to add only enough water to keep the mat submerged.

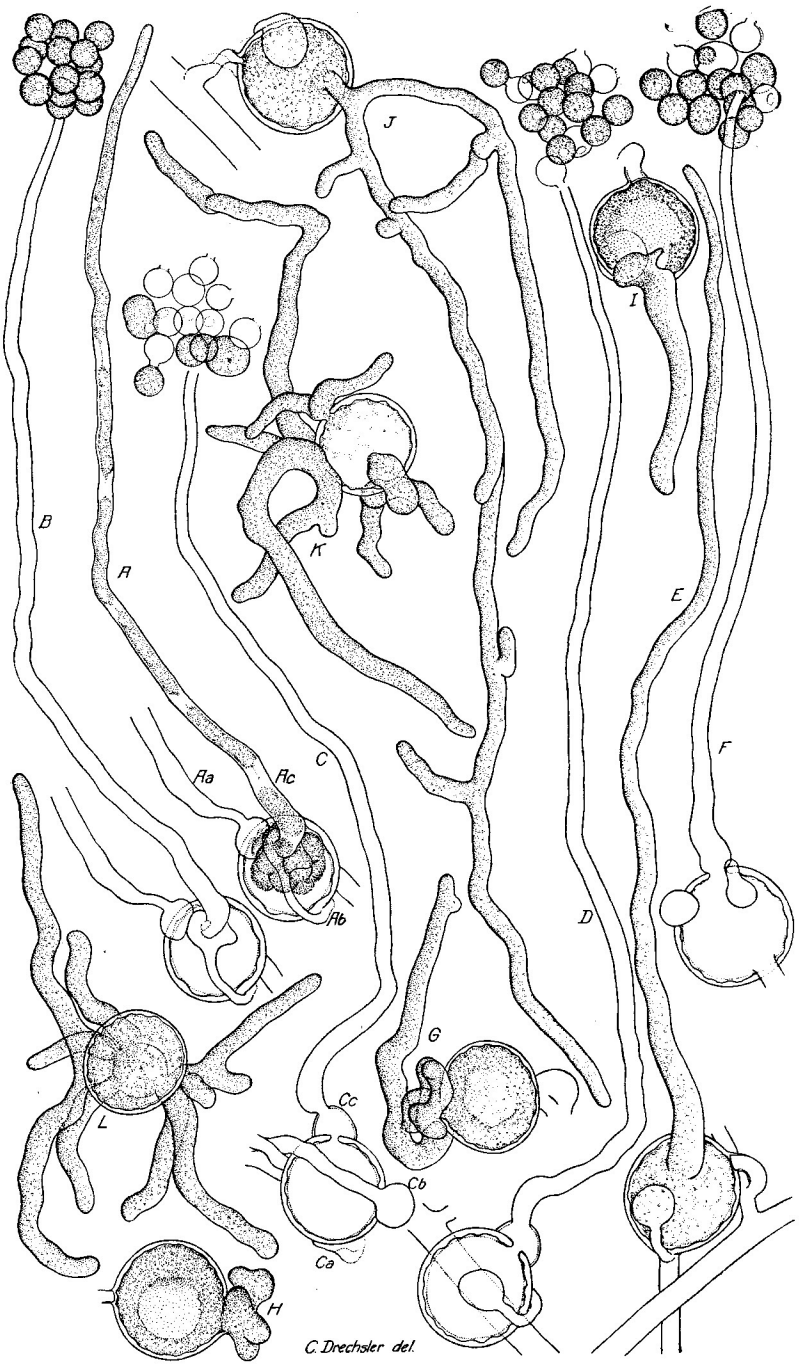
With young thalli at a temperature of about 20° C., evacuation of the sporangial filaments was found to begin about six to seven hours after washing was completed. As in the case of the germ sporangia, the internal developments follow the course described for congeneric species by other writers. It may not be superfluous however, to discuss certain matters which perhaps have not received adequate treatment hitherto, or which involve points in regard to which the fungus under consideration would appear to be at variance with its aquatic congeners.

For example, in the writings of most authors the distinction is made between vegetative hyphae and sporangia. While these structures are invariably said to be similar to each other in external morphology, the impression is conveyed that specialization in perhaps less obvious characteristics nevertheless obtains. Such a supposition, while conserving the analogy to other Saprolegniaceae, finds little support in the behavior of young vigorous thalli

EXPLANATORY LEGEND FOR PLATE 5

Oospores of *Aphanomyces euteiches* from 15 day old hard cornmeal agar cultures germinating in Van Tieghem preparations. × 470

A.—Germ sporangium 30 seconds before discharge
B.—Same sporangium 5 minutes later, after evacuation
C, D.—Evacuated germ sporangia showing utilization by germ hypha of aperture in oogonial wall produced by antheridium
E.—Oospore with single germ tube previous to separation of contents
F.—Evacuated germ sporangium
G to J.—Direct germination of oospores by production of hypha with ordinary type of branching
K, L.—Direct germination of oospore with close branching immediately after emergence of germ tubes



(For explanatory legend see p. 304)

subjected to the treatment outlined above. Under favorable conditions almost the entire thallus appears to become involved in sporogenesis, the individual sporangia often consisting of axial filaments from 1 to 2 mm. long and bearing from 6 to 10 well-developed branches. (Pl. 6, A.) The numbers of spores discharged from such extensive sporangial units is naturally very considerable, not infrequently running up to 300 or 400. (Pl. 6, *Bc.*) The individual sporangia are set off from adjacent sporangia, or still undifferentiated hyphae by partitions which may be plane or somewhat curved and are often inserted at the origin of a branch. (Pl. 6, A, D.) A vast number of zoospores may thus be produced in the course of a few hours, estimates made on such material often reaching several hundred thousand. It may be mentioned that the amount of growth observable after the washing away of the nutrient material is quite negligible, the possibility of extensive proliferation of new filaments originating as specialized organs being thus largely precluded. Nor do the sporangial units of such material differ in abundance of branching from the mycelium of the vegetative thallus in an actively growing condition, in spite of the customary characterization of the sporangia of congeneric forms as simple or rarely branching.

A distinction between hypha and sporangium might more plausibly be drawn when older material is used for the production of zoospores. Staling effects are here manifested in the degeneration of the contents of a large proportion of the hyphae. Sporogenesis is never prompt, a period of 48 hours usually elapsing before any considerable discharge occurs. The reason for such delay is evident on examination, when it will be found that the old hyphae are not functioning as sporangia; that these have, in fact, become evacuated, the contents having apparently been utilized in the production of new filaments in the central portions as well as at the margins of the thallus; and that sporogenesis is localized in the newly proliferated filaments, which indeed, show relatively little branching. Manifestly the potentiality of serving as sporangium is not limited to hyphae arising as specialized organs; but is inherent in any vigorous hyphae when given the necessary conditions. It may be remarked that Rothert (*15*) in his study of a congeneric form came to an entirely similar opinion in regard to this phase of reproduction.

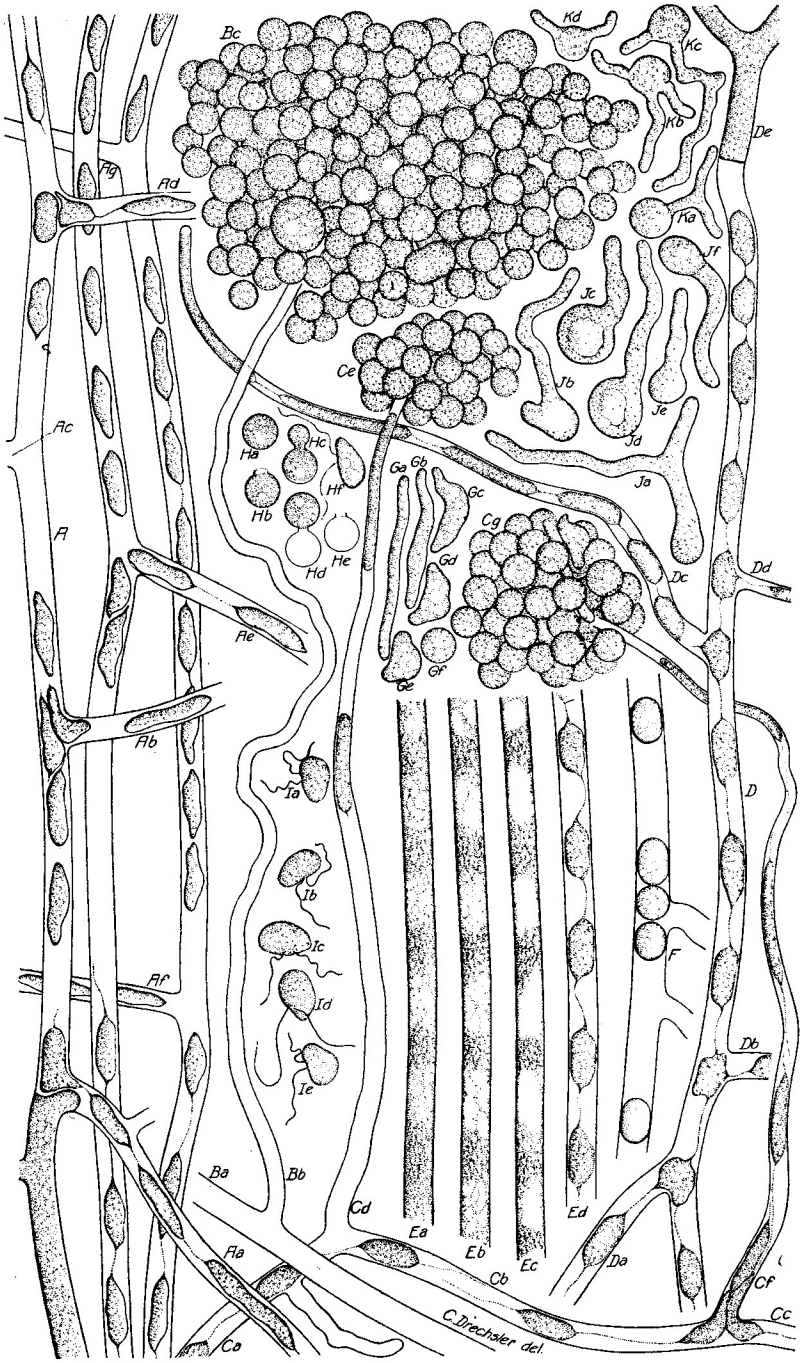
While the hyphae of the pea parasite in liquid culture, as within the host cell, tend toward evenness in diameter, pronounced local irregularities in respect to this dimension not being characteristic of the fungus, the terminal mycelial branches generally exhibit gradual attenuation toward the tip, the apical portion generally measuring 4 μ, or, more rarely, somewhat less. Evacuation of the individual sporangia very regularly takes place through these attenuated branches. As the protoplasmic masses pass from the larger hyphae into the constricted region they become considerably elongated and move at a proportionately increased speed. Thus a cylindrical mass 10 to 12 μ in length, occupying the lumen of a filament 9 μ wide and moving at a speed of about 35 μ a second, on reaching the distal portion of the evacuation hyphae will be found measuring 30 to 50 μ in length and moving more than 100 μ a second. In a few instances a considerable part of the discharge tube was found reduced to a diameter scarcely exceeding 3 μ. Discharge here offered the remarkable spectacle of zoospores drawn out into threadlike bodies, about 2.5 μ in diameter and 70 to 90 μ long, speeding along at the rate of approximately 300 μ per second.

The discharge of the zoospores from the freely branching sporangium presents an interesting complication. At

EXPLANATORY LEGEND FOR PLATE 6

Stages in asexual reproduction of *Aphanomyces euteiches*. ×470

A.—Portion of extensive sporangium drawn in three contiguous sections bearing six successive branches *ab-g*, evacuation of zoospores arrested after proceeding for some time
B.—*a*, Portion of sporangial unit with aggregation of approximately 350 zoospores
C.—*a* to *c*, Portion of sporangial unit, evacuated at beginning only through branch *cd*, then through both *cd* and *cf*, and finally only through *cf*
D.—Portion of sporangium 15 seconds previous to discharge through branch *dc*
E.—*a* to *d*, Successive stages in the conversion of hyphal contents into zoospores ready for discharge
F.—Zoospores rounded up within sporangial wall
G.—*a* to *f*, Successive stages in rounding up of cylindrical protoplasts, at intervals of 2 seconds
H.—*a* to *f*, Evacuation of encysted zoospore
I.—*a* to *e*, Motile zoospores treated with osmic acid and gentian violet, showing length and insertion of flagella
J.—*a* to *f*, Direct germination of encysted zoospores
K.—*a* to *d*, Germination of zoospores after swarming and second rounding up



For explanatory legend see p. 306)

each juncture of axial filament and branch two of the elements deliver their contents into the third. Where evacuation is not too rapid, the delivery alternates in a more or less orderly manner, one or several zoospores from one arm being followed by one or several zoospores from the other. When discharge is more rapid, some degree of disorder usually results, a number of zoospores from both sources often being squeezed into the efferent element so compactly that they may momentarily appear as a single protoplasmic mass (pl. 6, *Cf*) which further along in the course of the filament tends to become separated into its components.

As in other members of the genus Aphanomyces, the changes (pl. 6, *Ea*, C), resulting in the division of the mycelial contents into zoospores, leaves the individual protoplasmic masses connected by a delicate strand of tenuous material. (Pl. 6, Ed, D.) This strand can usually be seen without difficulty after evacuation has started, even when it has become further attenuated by lengthening of the intervals between successive zoospores. However, beyond the first juncture of two sporogenous elements the strands uniting the zoospores contributed by each of the elements are pressed against the confining mycelial wall by the interpolated zoospores contributed by the other, with the result that their continuity, if not actually destroyed, becomes at least very difficult to establish. After several junctures have been passed, so that the moving file of zoospores represents, perhaps, more than half a dozen interpolated series, it is certainly not possbile to make out a corresponding number of strands in the intervals. Usually only one or two can be made out in any particular gap, depending upon whether a strand can be demonstrated for one or for both of the two successive zoospores. (Pl. 6, D.) Rothert (*15*) in his study of an unnamed species of Aphanomyces believed that the strand persisted even where none was visible; that the fact of the escaping protoplasts being pointed at the ends indicated clearly enough the presence of a connecting medium capable of exerting a pull. To the traction exerted by the distal zoospores he assigned some importance in accomplishing the evacuation of the proximal ones. In the pea parasite, however, the zoospore while passing through the evacuation hypha are not always pointed at the ends, the anterior end especially being frequently well rounded as if no distorting pull were present.

A curious feature exhibited by the root-rot fungus which does not seem to have been recorded hitherto for any congeneric form is the discharge of the zoospores from a sporangial unit through plural evacuation hyphae. In the extensive units characteristic of vigorous young thalli converted to reproductive purposes, three or even four evacuation tubes have been found; and sometimes two of these may be close enough together that they can be observed simultaneously. Such an instance is represented in Plate 6, C. Evacuation here began through branch *Cd* and had proceeded briskly for about 15 seconds when the tip of branch *Cf* also yielded. For a number of seconds discharge occurred simultaneously with about equal rapidity from both tubes, the element *Ca* supplying tube *Cd*, while *Cf* was supplied from the element *Cc*. Soon discharge through *Cd* came to a standstill, and the element *Ca* contributed its zoospores through the intermediate portion *Cb* into *Cf*, the latter then being fed, as *Cd* had been previously, from both directions. A number of zoospores in the portion *Cb*, that had originally come from *Cc* and seemed bound at the time to emerge through hypha *Cd*, thus reversed their direction and were discharged through branch *Cf*. In other cases evacuation through two tubes was observed to take place simultaneously through both, now through one, now through the other, in repeated and apparently haphazard alternations. The entire process, with its reversals in direction resulting in the discharge of successive zoospores through separate evacuation branches, failed to suggest any considerable effectiveness of visible and possibly invisible connecting strands in determining the course of any individual zoospore.

On emerging from the mouth of the sporangium the spores are cylindrical in shape, straight or slightly curved. (Pl. 6, *Ga*.) Immediately, however, they begin to shorten up, and after passing through increasingly thick allantoid phases (pl. 6, *Gb* to *e*), appear at the end of about 10 seconds as perfectly spherical masses (pl. 6, *Gf*, although a certain proportion of irregular oversized individuals may usually be found. (Pl. 6 *Bc*, *Ce*, *Cg*.) The secretion of a thin peripheral wall follows very shortly. While in certain species of Aphanomyces the quiescent zoospores arrange themselves in a very regular hollow sphere having some considerable degree of coherence, in the form under consideration these bodies show little tendency toward definite orientation and relatively little co-

herence. They seem merely to accumulate promiscuously at the mouth of the sporangium in an irregular lump (pl. 6, *Bc*, *Ce*, and *Cg*), or where smaller numbers are concerned each individual may remain alone.

The development of motile zoospores from these encysted bodies takes place after a period varying in some observed instances from 1½ to 2½ hours. A papilla makes its appearance on the cyst, measuring between one-fourth to one-third the diameter of the latter. (Pl. 6, *Hb*.) At first scarcely discernible, it develops visibly in the course of several minutes, until it appears as a hemispherical protuberance. Suddenly the granular protoplasmic contents begin streaming into the papilla, the tip of which thus becomes inflated into a spherical vesicle. (Pl. 6, *Hc*.) Streaming is completed within approximately 10 seconds, the entire contents having then passed from the cyst into the vesicle. (Pl. 6, *He*.) The contour of the latter is not tangent to that of the empty cyst envelope, as these two structures are separated by a cylindrical isthmus, about 1 μ long, composed of the wall of the papilla. The whole process appears altogether analogous to the discharge of the sporangium of Pythium on a smaller scale, and, as in that genus, the discharged protoplast, after increasing distortional movements, finally develops motility and swims away, not as a number of zoospores, to be sure, but as a single zoospore, somewhat pointed at one end, with two cilia inserted laterally. (Pl. 6, *Hf*.) In material killed with osmic acid and stained with gentian violet, the flagella appear as threadlike structures approximately twice the length of the zoospore, one being somewhat longer than the other but the difference not being pronounced. (Pl. 6, *Ia* to *e*.)

After swimming about for a variable period the zoospore, as in other members of the Saproligniaceae, finally come to rest and round up. Under favorable conditions they germinate, producing from one to three germ tubes (pl. 6, *Ka* to *d*) capable of extensive development into a mycelium, either in artificial culture or in the tissues of a new host plant.

It may be superfluous to call attention to rather usual irregularities in development, cited frequently in the literature pertaining to related organisms. Among these might be mentioned the failure of the zoospores to escape from the sporangium (pl. 6, F) and their germination within the filament; the production of compound zoospores exhibiting two sets of flagella with erratic ineffectual movement, resulting from the germination of oversized and evidently dienergid cysts; and direct germination, without swarming, of encysted forms by the production of a single rather broad germ tube. (Pl. 6, *Ja* to *f*.)

TAXONOMY OF PARASITE

As suggested in another connection, it is not improbable that the parasite under consideration may have been observed by previous workers investigating the troubles affecting peas. Wittmack (*19*), in Germany, attached the binomial *Pythium sadebeckianum* to a fungus found occurring as oospores in the roots of peas, the presence of which was evidently associated with symptoms having some similarity to those described in this paper. Chiefly because the diameter of oogonia, as given by this author, —32 μ is in excess over those of any species of Pythium commonly attacking higher plants, students of the latter genus have been at a loss as to the identity of the German fungus. Judging from the widespread occurrence of the root rot parasite in diseased pea roots in the United States, it is at least likely that Wittmack, who apparently never observed sporangia in his material, may have assigned his fungus to the wrong genus. For quite similar reasons the same uncertainty attaches to Clinton's (*2*, p. 450–453) provisional identification of oospores observed during recent years in diseased pea roots in Connecticut as the oospores of *Phytophthora cactorum*. The identity of the forms observed by these writers with the fungus discussed in this paper at present can merely be suggested as a very fair possibility.

In this connection it may not be amiss to call attention to the somewhat unusual parasitic character of the rootrot organism when viewed in its taxonomic relation. While the Saprolegniaceae include a number of parasites affecting fish, only a few reports can be found of any members of the family attacking species of higher plants. In 1912 Sawada[5] published a paper containing a detailed study of *Achlya prolifera* (Nees) DeBary, as the cause of a rice seedling

[5] SAWADA, K. INVESTIGATION OF THE PADDY SEEDLING DECAY IN FORMOSA. Formosa Agr. Exp. Sta. Spec. Bul. 3, 84 p., illus. 1912. [In Japanese.] Brief statement of content in English, by W. H. Weston in Ann. Bot. 37: 347–348, 1923. Author's unpublished English résumé revised by W. H. Weston, deposited in Office of Cereal Investigations, Bureau of Plant Industry, Washington, D. C.

decay in Formosa. Nemec (*12*) in 1913 described from Prague as the type of a new genus an interesting fungus causing swellings on the roots of *Salix purpurea* under the binmoia *Jaraia salicis*. It is worthy of note that the fungi in these two instances effected their parasitism under conditions substantially aquatic, the willow plants attacked by *Jaraia salicis* being cultivated at the time in tap water in the greenhouse, while the destruction observed by Sawada presumably occurred while the host was kept flooded.

Within the genus Aphanomyces itself the tendency toward parasitism is moderately pronounced. Two of the eight described species, *A. phycophilus* DeBary and *A. norvegicus* Wille, attack species of Spirogyra and Zygnema (*1; 18*); two other members of the genus according to Coker, *A. stellatus* DeBary and *A. parasiticus* Coker (*3*), attack Achlya, and the same author discusses a variety of *A. laevis* DeBary that was found growing parasitically on diatoms and desmids. In these instances the hosts represent aquatic lower forms attacked under thoroughly aquatic conditions. The only account of a member of the genus, and, indeed, of the family, as far as the writers have been able to determine, attacking one of the higher plants under ordinary terrestrial conditions is contained in the report by Peters (*14*) of a form of Aphanomyces identified by him as *A. laevis*, as one of the three widely prevalent parasites responsible for root blight (Wurzelbrand) of sugar beets in Germany. Peters' publication is of particular interest, although in some pathological features the disease he investigated differs from the trouble affecting the subterranean parts of peas.

In considering the taxonomic disposition of the pea fungus, moreover, *Aphanomyces laevis* represents one of the two species deserving of special attention, the other being *A. helicoides* v. Minden. The remaining six congeneric forms are characterized by the presence on the oogonium of spines or of tuberculate irregularities, whereas the oogonia of the pea parasite, like those of the two species designated, are entirely smooth on the exterior. According to its author (*11*), *A. helicoides* is very similar to *A. laevis*, being distinguished chiefly by a strong tendency in the antheridial branches to wind about the oogonium, or about ordinary hyphae, or even about other antheridial branches, in close helicoid turns. Certain of Kasanowsky's drawings (*10:* Taf. X, fig. 1) represent some such condition, making it seem probable that this investigator was dealing with a type more nearly resembling the Swiss form than the one originally described by DeBary. Coker seems inclined to support Von Minden's own doubts concerning the validity of the helicoid habit as a specific distinction on the ground that a strong tendency toward such habit was exhibited by the typical form of *A. laevis*. Whatever disposition may finally be taken with reference to *A. helicoides* is, however, of minor concern here, for, as has been previously pointed out, no distinct tendency toward spiral growth has ever been found expressed in any of the cultures of the pea organism the writers have studied, although the branches bearing the sexual organs may be more or less involved after a more promiscuous fashion.

When the organism responsible for root rot of peas is compared with the typical aquatic form of *Aphanomyces laevis*, as revealed in the literature, a considerable measure of agreement becomes evident. The dimensions and interrelations of oogonium and antheridium and of the branches supporting them, as well as the dimensions of the oospores, correspond quite well in the two plants. A fair, even if not altogether perfect, agreement is evident in regard to size of zoospore and diameter of mycelium. The potentiality of the entire thallus to serve reproductive purposes by division into extensive branching portions functioning as individual sporangia; the discharge of the latter through one or several hyphae tapering regularly toward the tip to a diameter inferior to the diameter of the mycelium generally; the ready germination of the oospores, and that without resting period or application of special treatment—these features point to a distinction between the root parasite and the aquatic form, although it remains doubtful to what extent absence of their mention in the literature pertaining to the latter form is attributable to actual difference in its behavior or to shortcomings in our knowledge concerning it.

With regard to another character—namely, the thickness of the oogonial wall—it becomes almost impossible to entertain similar doubts. The accounts of *Aphanomyces laevis* given by DeBary, Humphrey (*5*), Kasanowsky, and Coker contain no reference to this feature, yet it is altogether unlikely that any of these investigators could have seen a structure of such extraordinary thickness without in some way referring to it. Indeed, on the contrary, Coker in his definition of the genus

Aphanomyces, recognizes a thin unpitted oogonial wall as a generic characteristic, presumably common to all species; and all of the authors mentioned figure the oogonial envelope of *A. laevis* as a thin membrane, not to be compared with even the least indurated of the homologous structures belonging to the parasite on pea roots.

In this connection it may be mentioned that the writers have cultivated an aquatic species of Aphanomyces with smooth oogonia, obtained through the courtesy of J. A. Lounsbury, who isolated it from water of Lake Mendota, where apparently it occurs in some abundance. This fungus, which as far as could be determined answers to the description of *A. laevis*, very obviously is not identical with the parasite affecting peas, developing a heavy matted, submerged growth on various agar media entirely unlike the arachnoid growth characteristic of the terrestrial organism. The oogonia are produced much more sparingly and always with the thin membranous wall made familiar by the figures of various writers. As the different isolations made from diseased peas compared with one another show unusual uniformity with reference to morphological characteristics, as well with reference to rapidity and character of growth on a variety of substrata, their incorporation in the same species with a form so evidently dissimilar as the aquatic plant could scarcely contribute to taxonomic clarity.

The identity of Peters' beet parasite remains problematical. This author undoubtedly was right in concluding that the measurements of his fungus, while somewhat different from those of *Aphanomyces laevis*, did not, in themselves, justify its recognition as a species distinct from the latter. It is interesting to note that he observed very marked variability in thickness of both oogonial and oospore walls, and as the thickness of these structures fluctuated together he believed that the fluctuations were contingent on the same environmental conditions affecting both. Thus in material containing oogonia so thin-walled that they were found collapsed, the oospores borne in them were provided with walls *only* 3 μ thick; while in other material oogonia with walls 1.5 μ thick contained oospores of which the envelope measured 5 to 6 μ in thickness. If the measurements of the oospore wall as given represent measurements of normal material, Peters' fungus would undoubtedly constitute a species different from any species of Aphanomyces hitherto described, as well as from the one described in this paper. However, grounds for suspecting that this is not the case are not wanting. In the pea parasite, for example, degenerate conditions of the sexual apparatus are far from rare even on the most favorable substratum like hard corn-meal agar; when less favorable media are used (potato or carrot decoction with 1.5 per cent agar, and also pea decoction in the absence of solid particles) degenerate conditions represent the overwhelming rule. The contents of the oogonium may become lumpy and degenerate before an oosphere is formed, or an oosphere may be formed but degenerate without forming a wall, or a wall may be formed but the contents degenerate instead of developing the normal structure. In any case swelling of the confining membrane is a regular concomitant of protoplasmic degeneration, whether this occur in oogonium or oospore, and in the absence of normal material such pathological modification might be mistaken for normal thickening. In view of these circumstances, it is hardly possible to decide definitely as to the morphological relationship of the two congeneric forms, both terrestrial in habitat and parasitic on a phanerogamic host.

It may also be stated that in the study of the pea organism structures involved in degenerative changes were not given weight; that the peculiarities of the oogonial wall submitted as normal were observed regularly in nature, as well as in culture, in individual female organs containing oospores provided with a wall of moderately and practically unvarying thickness containing a large, homogeneous, slightly eccentric ("subcentric" in the phraseology used by Coker) structure, surrounded by concentrically arranged granular-appearing bodies. The parasite, it is believed, may best be regarded as a new species. Because of the distinctive character of the oogonial envelope, the specific name *euteiches* is suggested.

TECHNICAL DESCRIPTION

APHANOMYCES EUTEICHES Drechsler n. sp.

Parasitic on the subterranean parts of cultivated peas (*Pisum sativum* L.), causing a destructive stem and rootrot capable of affecting the growing host at all ages.

Hyphae hyaline, branching at moderate intervals (20 to 150 μ) at angles approaching a right angle; 4 to 10 μ in diameter, the individual filaments not

abruptly varying in width; occurring in nature within cortical cells of host, in nutrient solutions as extensive nebulous translucent mycelia.

Sporangia in artificial culture arising by conversion of extensive portions of vegetative mycelium delimited by one or more septa; often including many ramifications; discharging through one or several (up to four) tapering branches, the distal portions of which measure usually approximately 4 μ.

Zoospores cylindrical, in escaping from evacuation branches becoming attenuated to vermiform bodies, usually 3.5 μ in diameter by 30 to 50 μ in length; forming spherical cysts at mouth of sporangium, measuring usually 8 to 11 μ in diameter, rarely up to 16 μ; diplanetic, the empty spherical wall being distinguished by a protruding evacuation tube 1 μ long by 2.5 to 3 μ in diameter.

Oogonium generally, if not always, terminal on a short lateral branch, from which it is delimited by a partition sometimes present as a simple septum, at other times as a columella-like structure protruding into the oogonial cavity; subspherical, measuring usually 25 to 35 μ in diameter; when mature exhibiting a heavy peripheral wall with smooth outer contour and sinuous inner contour, hence of irregular thickness, this dimension varying between 1 to 5 μ, generally between 1 to 2.5 μ.

Antheridia typically of diclinous origin, borne on a stalk frequently involved with the oogonial stalk, and often branching once or several times; measuring 8 to 10 μ in diameter by 15 to 18 μ in length, or when considerably larger often more conspicuously arched, somewhat lobulate, and becoming compound by the insertion of transverse septa.

Oospores subspherical or more rarely ellipsoidal owing to intruding columella-like septum; 18 to 25 μ (generally 20 to 23 μ) in diameter; provided with a wall of uniform thickness, this dimension varying between 1.2 and 1.8 μ (generally 1.5 μ); slightly eccentric in internal structure ("subcentric"); germinating without protracted resting period either directly by 1 to 3 germ hyphae or by production of a single unbranched sporangial filament usually 200 to 350 μ in length, in the latter event producing generally 13 to 18 zoospores, approximately half of which are delimited within oospore wall.

Collected repeatedly in diseased peas in Maryland, New York, Ohio, Indiana, Michigan, Illinois, Wisconsin, Montana, Idaho, Utah, and California.

PHYSIOLOGY OF PARASITE

GROWTH OF THE FUNGUS ON CULTURE MEDIA

The fungus has been grown on several of the common culture media, on all of which it produces more or less of a sparse white surface growth with little aerial mycelium. A few imperfectly formed oospores are usually found in older cultures on most media. However, on corn-meal agar, while the mycelial growth is rather less than on most substrata tried, there is a moderately abundant production of oospores which are capable of germination soon after they are formed. For this reason cultures on this substratum retain their vitality indefinitely, while those on other media soon perish. Semisolid media, such as cornmeal or oatmeal with varying amounts of water added also give good growth, but the oospores are less perfectly formed as the medium becomes more moist. The most useful liquid medium that has been found for the production of mycelium and sporangia is a pea decoction made by adding 10 to 20 peas, preferably of a wrinkled variety, to about 75 cc. of water in a flask. This liquid remains clear after sterilization in the autoclave, and mycelium growing in it can readily be washed free from all nutrient material. The fungus grows as a submerged tuft in this medium until it reaches the surface, over which it spreads as a mat. In about 7 to 10 days at room temperature oospores begin to form, and soon after most of the mycelium is found empty and dead.

EFFECT OF TEMPERATURE UPON THE DEVELOPMENT OF THE FUNGUS

GROWTH OF MYCELIUM.—The fact that this disease develops early in the spring indicates that the fungus must be able to thrive at comparatively low soil temperatures. In an experimental determination of the thermal limits within which the fungus will grow a semiliquid medium, composed of 1 part by weight of corn meal with 12 parts of water, was used. The growth of the fungus in the surface of this gelatinous material is so inconspicuous that small increments of growth can not be measured accurately, and while the limits of growth were determined, the optimum could only be roughly estimated. In cultures held at a series of constant temperatures in incubators vigorous growth occurred at 34° C., but no growth was observed at 37°. At the lower end of the series no growth was observed after six days at 8° to 10°,

while a growth of 1.5 cm. occurred at 9° to 11° in the same time. The optimum temperature for mycelial growth appeared to be between 15° and 34°.

FORMATION OF ZOOSPORES.—The temperatures at which zoospores were discharged from sporangia and became motile was determined as follows: A culture upon oatmeal mush six days old was washed as free as possible from its substratum, cut into fragments, and placed in sterile water in watch glasses which were distributed in incubators at a series of temperatures. The results are presented in Table I.

drops of sterile water to which spore-bearing mycelium with as little substratum as possible has been transferred. Germination has been secured at temperatures from 6.5° to 31° C. Germination with the production of zoospores occurs in 24 to 48 hours at 14° to 28°, a range that may be regarded as an optimum. Germination with the production of a few zoospores has been secured at a temperature as low as 9° to 10° in four days, while germ tubes which did not discharge zoospores developed in the same time at 6° to 7°. Thus the oospores germinate in practically the

TABLE I.—*The effect of temperature upon the formation and motility of zoospores of Aphanomyces euteiches and the germination of zoospores*

Temperature °C.	Time in hours					
	18	25	42	49	73	97
33–35		Groups.	Groups.	Disintegrating.		
21–22	Groups.	Groups.	Groups. Zoospores.	Inactive.		
19.5–21	Groups.	Groups. Zoospores.	Groups. Zoospores.	Inactive.		
15–16	Groups.	Groups. Zoospores.	Groups. Zoospores.	Groups. Zoospores.	Groups. Zoospores.	Inactive.
13–14		Groups.	Groups. Zoospores.	Groups. Zoospores.	Groups. Zoospores.	Inactive.
9–11			Groups.	Groups. Zoospores.	Groups. Zoospores.	Zoospores.
8–9.5					Groups.	Groups.
5–6.5						

NOTE.—The word "Groups" indicates the presence of groups of discharged encysted zoospores at the time of observation, and "zoospores" the presence of motile conditions.

GERMINATION OF OOSPORES.—The determination of the effect of temperature upon the germination of oospores has been rendered difficult by reason of the fact that no dependable method has been developed whereby a quantity of mature oospores can be produced at a given time. Oospores from the roots of peas have not been seen to germinate. Frequently cultures on corn-meal agar three weeks old will germinate readily when placed in water, but sometimes only one culture among many made at the same time will respond in this way. Spores on the scant aerial mycelium which can be separated from the substratum usually germinate sparsely, while spores on or in the substratum often begin germination as soon as mature. In spite of the difficulties which have arisen from these irregularities in behavior, fragmentary records have been made which appear to delimit closely the range within which germination occurs and the optimum. Germination has been studied in hanging

entire range of temperature at which vegetative activity occurs, and the discharge of zoospores from the sporanges produced by the germination is affected by temperature in exactly the same manner as in the previous experiments where sporanges derived more remotely from oospores were used.

PATHOGENICITY

PENETRATION

A study of lesions in early stages occurring in the field or in greenhouse inoculations rarely reveals the first penetration of the fungus into tissue. In roots entry is accomplished without discoloration or visible indication of the fact, and later the entering mycelium, devoid of contents, is practically invisible. In the base of the stem a yellow discoloration in a few epidermal cells often indicates a point of entry. The invasion of the base of the stem is usually accomplished by the advance of the fungus upward from the root

rather than from direct entry. When peas are sterilized superficially and grown under sterile conditions in agar upon which the fungus is also growing, penetration of roots can then be found readily in thin razor sections of the root surface. The mycelium passing along the root surface turns inward at the junction of two epidermal cells and passes readily into one of these cells, whence it advances into an adjoining cell or into underlying tissue. (Pl. 3, B.) The point of entry and passage from one cell to another is not marked by any conspicuous constriction of the fungus strand. Invaded tissue softens immediately even in the absence of bacteria, indicating perhaps the production of an enzyme which softens cell walls and aids in fungal penetration.

RELATION OF SOIL TEMPERATURE AND MOISTURE TO THE DEVELOPMENT OF THE DISEASE

EXPERIMENTAL STUDIES

Among the many diseases of roots of plants few show in the field a greater degree of apparently erratic irregularity than the one under consideration. Even in fields which are very severely infested the disease is usually much worse in spots, and at its first appearance it occurs in irregular areas which are frequently coincident with the more moist soil, but at other times are not clearly related to any obvious soil differences. It appears that the occurrence of this disease is greatly influenced by environmental conditions. In its seasonal development temperature is undoubtedly a limiting factor, and in its local occurrence the field evidence suggests soil moisture as the more important limiting factor. In order to gain exact experimental evidence of the relations of these factors to the development of the disease, several series of plantings were made in the Wisconsin soil temperature tanks where both temperature and moisture were controlled within narrow limits. Two of these series will be reported in detail.

The soil chosen was a sandy loam from a field in which peas had never been grown, and as a further precaution against the presence of root-destroying fungi it was treated with formaldehyde a month before use and subsequently thoroughly dried until no trace of this sterilizing agent was detected. The soil was inoculated one week before planting by mixing thoroughly with small fragments of roll cultures of an isolation of this fungus which had shown very active parasitism in previous tests. The viability of the oospores in these cultures had also been previously demonstrated. The inoculated soil was packed in 6-inch cans, near the bottom of which an irrigation apparatus was arranged to permit the addition of water without flooding the surface of the soil. On November 18, 15 Alaska peas were planted at the depth of about 2½ inches in each can. Six cans were placed in each of a series of tanks, which were maintained at 12°, 18°, 24°, and 30° C., respectively. The moisture present in the soil at planting was 15 per cent of its dry weight, or about 35 per cent of the moisture-holding capacity of the soil, previously determined to be 42 per cent of its dry weight. Two of the cans in each tank were maintained at the original soil moisture, while in two others the water was increased to 60 per cent of the moisture-holding capacity of the soil, and in the remaining two cans it was raised to 80 per cent of the moisture-holding capacity.

Inoculation in this instance was so effective that soon after they had emerged from the ground a few of the plants decayed and wilted down, whereupon they were removed. At the conclusion of the series, on December 18, the remaining plants were removed from the soil, and record taken of those showing decay of the base of the stem and of the roots. (See Table II.)

From these data it appears that the fungus was restrained little if any in its parasitic activity toward peas at 30° C., which is within 3° of the upper limit of soil temperature at which peas can be induced to make an approximately normal growth. In soil with the highest moisture content the progress of the disease does not appear to be favored as much as might be expected. Since some degree of infection took place even at the lowest soil temperature maintained here, and the activity of the parasite at the lower range of temperature is of greater interest, a new series was started December 19, with the purpose of exploring more thoroughly the range of temperature which occurs in the field at the time when the disease normally appears.

In the second series the soil used before was mixed, dried somewhat, and placed in cans. The moisture in this soil was found to be 36 per cent of its moisture-holding capacity, and adjustments were made to 60 and 80 per cent to correspond with the earlier series. (See Table III.)

TABLE II.—*The pathogenicity of Aphanomyces euteiches to Alaska peas at the series of soil temperatures and in soil at the three conditions of soil moisture indicated (series of November 18, 1923)*

Temperature in °C	12°			18°			24°			30°		
Per cent moisture-holding capacity of soil	35	60	80	35	60	80	35	60	80	35	60	80
Number of plants Dec. 1	25	27	25	27	26	26	25	25	24	28	26	27
Number of plants wilting and removed at date indicated:												
Dec. 1								1	2			3
Dec. 2								2	4			2
Dec. 6							2	1	7		7	6
Dec. 8					1	2		3	1		7	2
Dec. 13					1	2	8	5	4		4	2
Dec. 17				3	2	1	5	2			2	1
Total number of dead plants				3	4	5	15	14	18	(a)	20	16
Number of plants with decayed stem bases Dec. 18	3	9	3	10	19	11	10	4	(b)	1	3	8
Total number of plants infected	3	9	3	13	23	16	25	18		1	23	24
Healthy plants, Dec. 18	25	21	25	15	5	8	0	5	(b)	12	4	1

[a] One of the two cans at this temperature and moisture developed a leak and was discarded.
[b] Data missing.

TABLE III.—*The pathogenicity of Aphanomyces euteiches to Alaska peas at the series of soil temperatures and at the three conditions of soil moisture indicated (series of December 19, 1922)*

Temperature in ° C	10°			12°			15°			18°			21°			24°		
Per cent moisture-holding capacity of soil	36	60	80	36	60	80	36	60	80	36	60	80	36	60	80	36	60	80
Number of plants Jan. 5							27	36	32	34	34	28	38	24	32	39	31	28
Number of plants wilting and removed at date indicated:																		
Jan. 2												1			1		1	3
Jan. 5														2	1			
Jan. 9									1			5		4	3	4	1	13
Jan. 12									2		1	1				6		3
Jan. 19									3	5		4		1	3	7	2	4
Total number of plants killed									6	5	1	11		7	8	17	4	23
Number of plants diseased Jan. 30		1	1	2	0	2	17	8	10	22	19	6	10	5	17	12	7	5
Total number of plants infected		1	1	2	0	2	17	8	16	27	20	17	10	12	25	29	11	28
Healthy plants Jan. 30		29	20	30	32	19	14	25	16	6	13	10	24	13	8	7	17	0

The soil from this experiment was used for a third series in the temperature tanks, in which the method was modified for the purpose of learning whether infection occurring later in the development of the plant would produce less wilting than early infection. The soil was dried until it contained 30 per cent of its moisture-holding capacity, mixed thoroughly, and placed in cans. Peas were planted on February 10, and the entire series was kept at a soil temperature of 12° C. until February 24, when most of the plants had emerged, though not all had assumed an erect position. Temperature was adjusted to 15°, 18°, 21°, and 27°, and water was added to bring the soil up to 60 per cent of its moisture-holding capacity. New inoculation of these plants was then made by pouring a suspension of zoospores around the bases of all plants, and the surface soil was kept moist for the three following days. In five days a few plants at 27° showed infection at the surface of the soil, from which they died later; but there was so little infection at the lower temperatures that the results of this series are not

presented in detail. The paucity of infection in this experiment is not easily explained.

The data presented from the two previous series indicate clearly that though occasional infection may take place through practically the entire range of temperature at which peas will grow, infection is not abundant nor does invasion proceed rapidly at a temperature below 15° C. The optimum temperature for infection is between 15° and 30°. Within this range infection appears to be approximately uniformly abundant, but at the lower temperatures there is a retardation in rate of progress of the fungus through the plant that is even greater than the retarding effect of temperature upon root growth as it occurs under the conditions of experiment. It is probable, however, that in the field in the spring with longer days root growth is more rapid at lower temperatures than under the conditions of experiment, and if that is the case the apparent retarding effect of low temperature upon the progress of the disease in the field will be greater than is shown here.

The effect of soil moisture upon infection in these experiments was not as great as anticipated. In fact, they do not give any decisive indication that soil moisture within the limits used in these experiments is a factor at all in determining the amount of infection. Although infected plants in the more moist soil uniformly begin to die a little earlier than those in drier soil, the number is in most cases approximately equal.

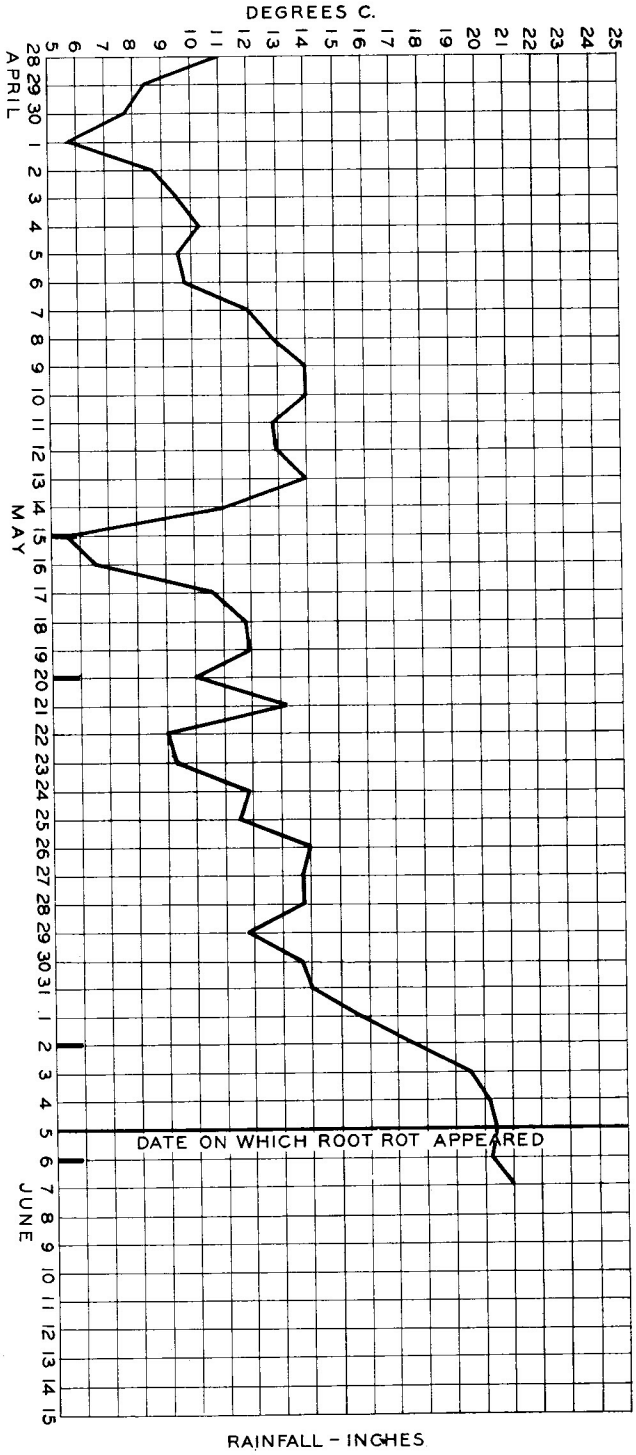
Further consideration of these experimental data may serve to bring out more clearly their limitations when an attempt is made to interpret field experience in their light. In these experiments it may be assumed that an approximately equal amount of viable fungus mycelium was brought in contact with the root systems of plants. The number of infections obtained is an indication of the ability of the fungus to infect under these conditions. It is possible that in the field soil moisture exerts an important effect, not only upon infection, but upon the survival of the fungus from year to year. The favorable effect of wet soil upon the disease may be due in large part, if not wholly, to the favorable environment which moisture provides for the fungus in its resting condition as oospores, or in a possible saprophytic life in the soil. Such an effect of soil moisture increasing the active vegetative growth of the fungus in the field may account for the association of disease with wet soil, rather than any effect of moisture upon the penetration of the plant by the parasite.

These experimental data should be compared carefully with observations of the development of the disease in the field on infested land to determine whether the inferences which may be drawn from the experiments are supported by practical experience. Unfortunately, there is but a limited amount of exact field observation which will serve for comparison. The development of the disease in experimental plats at Madison, Wis., on ground thoroughly infested with the fungus has been followed more closely than elsewhere. Planting in this plat was made in 1922 and 1923 on the same date, April 28. In 1923, the roots from these plants remained free from any trace of disease until May 30, when a very few rootlets showing typical decay due to Aphanomyces were found. On June 5 the entire root systems of these plants had begun to decay from a great number of infections distributed from the surface of the ground to the deepest roots. In this brief space of time abundant oogonia and a few mature oospores were formed.

This sudden appearance of disease followed promptly after the first favorable period of temperature and moisture that had occurred since the crop was planted. (Fig. 1.) The month of May of that year was exceedingly dry. The only rains sufficient to wet the soil for a brief period had fallen on May 15, 19, and June 2. The first rain occurred when the plants were emerging from the ground, and was followed by cold weather, the soil temperature [6] at a depth of 2 inches falling at night as low as 1° to 7° C. The second rain on the 19th was followed by weather hardly warmer, the mean soil temperature for the next five days ranging from 9° to 13.3°, a temperature at which oospores would germinate very slowly

The temperature rose very slowly after this date until June 2, when a heavy thunder storm gave a precipitation of 0.92 inch, wetting the soil in the plats to a depth of about 5 inches. After this day the minimum soil temperature did not fall below 15° C., and the mean daily soil temperature rose in the following five days from 17.5° to 20.5°. This coincidence of moisture with high soil temperature furnished optimum conditions for the

[6] The writer is indebted to J. G. Dickson for the use of soil temperature records quoted here.



1.—Average daily soil temperature in experimental plats at a depth of 2 inches and precipitation at Madison, Wis., from April 28 to June 15, 1923.

development of the disease which appeared with such remarkable suddenness. In fact, the period of time elapsing between the rain and such extensive invasion of the plant was so brief that it seems unlikely that the rain started the fungus to active vegetative growth from oospores, but rather that it had achieved some development during the period of dry weather. A heavy rain on June 6 with continued high soil temperature maintained favorable conditions for the development of the disease for a brief time until it was checked by returning drought, which lasted until the end of the growing period of the crop. It may be added that the shower of June 2 was local, not extending to the entire pea-growing region of the State, and that the general rain of June 6 was in many places the only precipitation during the entire season which wet the soil for even a brief period after temperature permitted infection of plants. Thus the fact that conditions suitable for infection did not occur until comparatively late in the season and were maintained for so brief a period reduced the amount of infection and injury from this disease to a very small amount that year.

In 1922 the onset of the disease was not as sudden or as severe. Some infected plants were found first on May 29, and thorough decay of entire root systems had occurred on June 2. Unfortunately no soil-temperature records were kept at Madison during the month of May of this year which can be compared with the records of 1923 previously cited. However, the Madison Monthly Meteorological Summary shows that the warmest weather of the month occurred May 9 to 12 during a dry period. Precipitation of over 2 inches occurred May 23 to 26, followed by six days with an average mean daily air temperature of 17.7° C., an average which is over 3° higher than of the six days after the rain of May 19, 1923, when no infection occurred. If this increase in air temperature was accompanied by a corresponding increase in soil temperature, a safe assumption since the weather record showed that they were days of almost uninterrupted sunshine, it would be just sufficient to provide a favorable temperature for infection, as determined in controlled experiments. It will, however, be necessary to accumulate many exact observations of the date at which disease appears and records of soil temperature and rainfall preceding the appearance of disease before it will be possible to predict accurately from weather records alone when the disease may be expected to appear; but it seems not unlikely that the time will come when such prediction can be made with precision.

RELATION OF THE PARASITE TO THE HOST

METHODS AND RESULTS OF EXPERIMENTAL INOCULATION IN THE FIELD

It will not be a matter of surprise that a fungus with a mycelium which is converted into perishable zoospores very readily and which forms oospores apparently under such restricted environmental conditions is not only limited in its ability to persist in soils, but, when present, is not always in active condition ready to invade plants. To secure such early and complete ruin of plants from inoculations with pure cultures, as often occurs in the fields, is not always an easy feat. The first difficulty which was encountered, though it appears to have been quite exceptional, deserves passing note. The first culture of this species, which was isolated, proved in the preliminary inoculation experiments to have so slight a degree of pathogenicity that the senior writer was misled and was delayed in recognizing the true cause of this disease. There is no apparent reason for the lack of pathogenicity of this isolation, and none of the seven later isolations which have been used in inoculation experiments have been thus deficient, though there appears to be a slight difference in virulence between some of them.

For greenhouse experiments inoculation of the soil by mixture of fragments of culture on corn-meal agar which have many mature oospores is the most satisfactory. Although such soil does not often give more than 50 per cent severe infection when first used, on a second planting will usually give better results, especially if care is taken to prevent drying out at any time. The use of zoospores in soil, either at the time of planting or poured on the soil when the plants have emerged, is uncertain. When plants are grown in sand, however, zoospores poured around young plants have given very thorough infection, provided great care is taken to keep the sand saturated for a time after inoculation is made.

Field inoculations have been made only at Madison, Wis., in 1923, a year so dry that it was everywhere unfavorable for the development of the disease. Inoculum was prepared in

several forms—earth previously inoculated with cultures in greenhouse experiments, cultures containing oospores planted with the seed, and zoospores applied at planting and at various times later. It seems inadvisable to present detailed results obtained thus in a single unfavorable season. Suffice it to say that some infection was obtained by all methods, but that the effectiveness of the method seemed to depend more upon the rainfall and temperature at and immediately after inoculation than upon the method. Zoospores poured upon the soil about Alaska peas breaking ground just before a rain on May 17 gave as high as 86 per cent infection, and when the soil was removed, exposing about 2 inches of the stem before the spores were distributed, every plant became infected, as evidenced by examination of the roots. Conspicuous decay following this infection of roots did not begin in any case, however, until the disease developed in plants growing on naturally infested soil. This simultaneous development of disease in plats whether infested naturally or artifically suggests that soil temperature controlled this development regardless of the time and manner of infection. All diseased plants in plats inoculated artifically grew to normal maturity and showed no conspicuous evidence of reduced vigor.

In contrast with these results of artificial inoculation were some data on the effects of natural inoculation in a similar type of soil not far removed where peas had been grown repeatedly for 7 years. Double rows of peas one rod long were planted on the infested soil, and extensions of these rows upon ground unused for peas were likewise planted with 400 peas to each rod. Unfortunately the disease had spread somewhat to the new land, so that many roots became infected, especially those of the susceptible variety, Surprize. However, the peas on the infested land showed early in the season all the symptoms of severe root rot, whereas those on the new land showed little indication of disease except at the ends of the rows adjoining diseased ground. Careful examination of plants in these plats during the season failed to reveal any other disease than that caused by Aphanomyces. The yields of some of these rows are given in Table IV.

The striking contrast between the results of natural infection and of artificial inoculation in the field plats is certainly not due to actual killing of the plants and demands explanation.

TABLE IV.—*Yields of dry peas in one rod of double row of peas upon soil heavily infested with Aphanomyces euteiches compared with yields upon adjoining ground where peas had not been grown previously. Planting was made April 28, 1923; 400 peas were planted to each rod of double row*

Variety	Infested land		New land	
	Number of plants growing	Yield	Number of plants growing	Yield
		Grams		*Grams*
Alaska	333	250	367	420
Do	369	210	378	393
Surprise	237	60	267	167
Do	297	71	292	210
Alaska	364	172	346	328
Do	360	125	358	318

Very careful and frequent examinations were made of the roots in all plantings. From these examinations it was found that the number of infected roots of the plants inoculated artificially were few and restricted to the region in which the inoculum had been applied, whether above, below, or near the seed. Under the conditions obtaining during this season, even the complete destruction of the cortex of the base of the stem rarely injured a plant greatly, provided most of the roots remained free from invasion. Although in artificial inoculations trenches in which peas were planted were drenched with zoospore suspension at the time of planting, infections were few and only near the seed. No method of inoculation after the plants had started growth seems to have filled the soil very thoroughly with the parasite in such condition that it was ready to invade the plant at a large number of points at once when favorable conditions for such infection occurred. It appears from field observation described elsewhere, as well as from these experiments, that this disease can produce conspicuous injury to top growth only when plants are either infected under favorable conditions for the development of the parasite when the plants are small or when they suffer a large number of infections at a later stage of growth. In these artificial inoculations the fungus was not distributed deeply and thoroughly enough in the soil to make possible a great number of infections when the brief favorable period for infection arrived; so brief was the favorable period that inoculations of young plants made when it

arrived did not have time to give a great number of infections before dry weather checked the growth of the fungus and prevented further infections and perhaps checked the progress of the fungus through the host tissue.

DISSEMINATION OF THE FUNGUS

The character of the fungus described in the previous pages points clearly to certain obvious methods whereby it may be distributed. Any transfer of soil from diseased fields will carry the parasite with it. In localities where soil from old fields is used to inoculate new fields with the bacteria which produce the beneficial nodules on the roots the parasite also will be carried if it is present. An excellent example of the harm which may be brought about in this way was seen in 1922 in Maryland. Soil from an old pea field in which the present crop was withering before maturity because of this root rot had been used to inoculate several neighboring fields used for peas for the first time. In every one of these fields thus inoculated individual plants scattered uniformly were dying, and others were infected, not enough in all to reduce the yield of this first crop appreciably, but enough to infest the soil so thoroughly that the failure of future crops was assured. Since transfer of soil from old fields to new may carry any of the known pea diseases which happen to be present, this practice is so often the cause of ruin to crops which are grown two or three years after the fields have thus been inoculated with disease that it appears to produce on the whole much more harm than benefit.

The flow of surface water over adjacent fields is an excellent conveyor of the fungus to new ground. The course which floods have taken across fields is sometimes indicated by areas of blighted peas. Irrigation water may be an excellent conveyor of the parasite. When the soil of an infested field is light and blown by wind, it may be a source of infestation for a large territory. In this manner a few centers of infestation are believed by R. E. Vaughan, who is familiar with the territory, to have ruined the entire area of the Truax Prairies in Eau Claire County, Wis., for pea growing. In the spring of 1922 some early plantings of peas were visited at Rochelle, Ill., when a large part of the land surface was being prepared for planting. A violent wind was drying out the surface soil so rapidly and picking up so much dust from it that the whole level country seemed covered with a fog clinging close to the ground. The distribution of spores with soil particles from a few infested fields in this locality under such conditions may account for the occasional diseased plants which are sometimes found when peas are grown for the first time in this vicinity, and the rather rapid subsequent increase of infestation.

There is a widespread opinion among growers that this disease is often introduced into new fields by the use of infected pea seed grown in diseased fields. This opinion, however, does not appear to be justified. Since the fungus does not enter the plant above the ground, it never reaches the seed and could only become attached to the seed in particles of dust from the soil. Even though this might conceivably happen, it appears to be a remote possibility, and, in any case, very few of the spores which could withstand drying would be present, so few that they would almost certainly escape detection by any experimental method that could be used and, therefore, so few that the disease which they would produce would not become conspicuous until several successive crops of peas have been grown. Although it is impossible to say that new infestation never takes place in this manner it appears to be at least a rare occurrence.

CONTROL MEASURES

CROP ROTATION

Although from early times the tradition of pea growing warns of failure which will attend the planting of peas on the same ground repeatedly, and English experience has developed a rotation of five or six years' duration as an insurance against disease, there are, on the other hand, so many advantages in intensive culture of the crop for the commercial purposes for which it is chiefly grown in the United States that rotation has usually been adopted only when the failure of the crop made this course necessary. A long rotation has been found to have a number of disadvantages of more or less importance. In some localities experience has abundantly shown that the second crop of peas on new land thrives better than the first, and the third may produce a better crop than either of the preceding. Peas grown for canning can often be produced more cheaply in a limited area close to the canning plant than in the much larger territory required by a long rotation. In the pea-seed-growing dis-

tricts of Montana, Idaho, and other Western States, this crop is sometimes more profitable than others, and since the suitable land area is limited the temptation to grow peas repeatedly is strong. Under the pressure of these economic reasons which favor the intensive culture of peas, many questions regarding rotation have become of vital interest to growers—how long peas may be grown before rotation is necessary, how short the rotation can be made with safety, and whether perchance new pea-growing districts may be found from which disease can be excluded or in which it will not develop.

Rotation as a control measure in combating pea diseases is generally discussed, not as a remedy for one of the many diseases of foliage and of roots, but as a control measure suited to secure relief from all of them in so far as they occur in the locality. Therefore, even though it is properly outside the limits of this paper to discuss more than the rotation that is needed to secure protection from the root rot caused by *Aphanomyces euteiches*, it will be futile to recommend a rotation which is suitable to protect from this disease alone. In the following pages this matter has been kept in mind, and, although it will be shown that a suitable rotation for peas is a local matter which must be determined with reference to the soil and climatic conditions existing there, it may be said that it appears generally true that any rotation which will control this form of rootrot will be adequate to hold other diseases sufficiently in check, at least in so far as rotation can control them.

First of all, it may be said that even when peas are grown intensively, an adequate inspection of fields can always or nearly always detect the presence of disease before it has begun to reduce yields, so that the crop can be discontinued on infested soil before losses have occurred. New soils have never been found heavily infested with the fungus unless perchance there is a badly infested field near by. The disease first appears under usual conditions in small areas, whence it spreads in successive years to the rest of the field. Usually the disease does not reach serious extent until the second year after diseased plants can be readily found. Careful inspection of fields can be made an adequate safeguard against loss wherever there is economic advantage in growing peas intensively, provided peas are not returned to land on which disease has appeared for many years.

It is very difficult to predict how soon disease will begin to appear upon any given tract of land. Experience shows enormous variation in this regard. Sometimes the third successive crop is rendered unprofitable, while neighboring fields may grow peas for 5 or 6 years successfully, and there are instances known both in Wisconsin and in irrigated districts in which peas have been grown in fields nearly every year for 10 or 12 years without a single failure. There are two possible explanations for such differences in time before the appearance of disease; either the parasite may have been absent originally from the fields which grew peas without disease for the longer period, or some soil condition may have prevented its rapid distribution and development. There is some evidence which indicates that the first condition furnishes the explanation for the late appearance of disease in some districts where peas have not been grown previously. There is a widespread belief among growers that fields in a new district will grow peas successfully much longer than fields in a district where root rot is known. That this is not always true is shown by the following instance. In the summer of 1922 a canning factory in Wisconsin far north of any locality where peas have been grown intensively was growing its third crop of peas. One field had been planted the three years in succession, four had been planted two years, and the remaining fields were on new ground. When an examination of these fields was made, about 25 per cent of the plants in the field producing its third crop were found dying with root rot, a few small groups of infected plants were found in each of the fields producing the second successive crop; while no diseased plants were discovered in any of the several other fields that were carefully searched. Although it may be true that the absence or scarcity of the fungus from some localities may explain in part the tardy appearance of root rot, it is certainly difficult to obtain adequate evidence in support of such an opinion.

On the other hand, there is abundant evidence that the disease develops much more rapidly and severely in fields upon some soil types than in neighboring fields upon other types. For instance, fields on Superior red clay in Wisconsin appear to develop root rot earlier and more severely than on any other type of soil. A minor factor that may contribute to the severity of the disease upon this type of soil may be due to the fact that, because it can not usually be worked in the spring

as early as lighter types, planting is delayed, so that the plants are younger and more readily destroyed when conditions favorable for the disease develop This condition does not furnish an adequate explanation for the prompt appearance of the disease with intensive culture on this soil, however. Another soil type upon which the disease develops with almost equal severity, though usually at a later stage in the development of the plant, is a very sandy soil in the trucking district south of Baltimore, Md. Although it is not obvious at first that there is any common condition in these extreme soil types that causes them to be favorable for the development of the disease, nevertheless observation of conditions in these districts and in others which will be cited later has led to the conclusion that it is the retention of water in both soils for a long period after rains that furnishes the favorable environment for the fungus. The Superior red clay is rather impervious to the passage of water and when once thoroughly saturated retains surface water in hollows and in the soil structure more persistently than do lighter types of soil. This offers conditions favorable for the germination of the oospores of Aphanomyces which may be present and perhaps for the production of zoospores. On the other hand, the sandy soil referred to is underlaid with an impervious subsoil, which, in more level fields, holds standing water in the lower portion of the sand for several days after heavy rains, thus providing a similar environment for the development of the parasite.

From this it appears that soil which is naturally retentive of water or in which water is held by reason of its relation to impervious layers furnishes the most favorable condition for the development of the disease. Such a supposition is rendered very plausible by the character of the fungus and is supported by other evidence, gathered not only in humid regions but in irrigated districts as well. The best examples of the relation of soil type and method of irrigation to the development of root rot have been found in the Gallatin and Paradise Valleys in Montana and near St. Anthony, Idaho. In the Gallatin Valley in 1921 this disease was found for the first time in a small field which appeared to have been overirrigated. The condition of the roots indicated that the infestation had developed after the plants were nearly grown, and although the root systems were thoroughly rotted the yield from this field was not apparently reduced. Since this was the first observed instance of the development of the disease in this valley, and it offered an excellent opportunity to obtain evidence which would indicate the severity which the disease might attain here, arrangement was made with the canning company for whom the peas were grown to have a portion of this field replanted with peas the following year. Such a field would not have produced an average crop of peas in a nonirrigated territory. Unfortunately the senior writer was unable to revisit this valley in the following year, but the canning company reported a normal yield of peas with no conspicuous evidence of disease. When the valley was revisited in 1923, a careful search of fields reported to have been used for this crop almost continuously for 10 or 12 years yielded but a single plant which was unmistakably diseased by this fungus. So far as this valley is concerned, there is no doubt that the fungus is present and that there are abundant facilities for its rapid distribution; yet, save possibly in cases of overirrigation, it appears that it is unable to become injurious. In contrast with conditions in this valley, fields in the Pine Creek district of the Paradise Valley about 25 miles distant from the Gallatin Valley were found thoroughly infested and greatly injured by root rot both in 1921 and 1923. Peas have been grown extensively in both valleys for at least 12 years under very similar climatic conditions. Whether a difference in soil structure or difference in the practice of irrigation has produced conditions favorable to the development of the disease in the Paradise Valley is not unmistakably apparent from observation.

No soil survey has yet been made of the Paradise Valley whereby soil formation there can be compared with that in the Gallatin Valley. There are, however, conspicuous differences in topography which suggest a difference in origin of the soils and a structure more retentive of water and conducive to seepage from higher levels in some fields of the Paradise Valley.

If the water-holding capacity of soil or the retention of water in the soil is so necessary for the development of root rot, we may ask again whether the apparent immunity from disease which some Wisconsin fields have shown may not be due to soil conditions which prevent the development of the parasite. Before a final answer is given to this question a large number of fields which

are known to have enjoyed this freedom from disease must be examined. In a conspicuous instance of such immunity examined recently, the subsoil of the field was found to be sand, which afforded admirable drainage. It may be that a limited amount of land will be found outside of irrigated territory on which peas may be grown intensively for a long time without trouble from root rot. However, most soil which has drainage and texture open enough to secure immunity to disease will probably be found too dry and infertile to produce large yields from a plant with a comparatively small root system like the pea.

Since there is a large amount of valuable land now incapable of producing profitable crops of peas because of infestation with root rot, the question is often asked how long the fungus remains in the soil, and if a rotation can be devised that will make possible the utilization of this land in the future. Since there is no experimental evidence which will aid in answering these questions, it is necessary to seek the experience of growers who have abandoned land for pea growing because of disease for varying periods of years. Experience of this kind is always open to question because one can not be sure that this root rot and not some other disease was the cause of the crop failure, both before and after the period in which the land was used for other crops. Notwithstanding such doubts, there appear to be two well-attested instances in Wisconsin in which fields failed to produce healthy peas after being used six years for other crops following a complete failure from rootrot. The writer has seen two fields of peas ruined by root rot after an interval of four years following a failure and another field in the same locality producing healthy peas nine years after a failure, presumably from this disease. In contrast with this Wisconsin experience, healthy fields of peas have been found in sandy soil in Delaware where peas failed four years earlier from disease which may have been root rot. From these instances and other experiences which might be cited it seems that in the heavier soils the parasite may persist for at least six years after it has once become abundant. In light soils its period of survival may be shorter. Probably no other fungus parasite of peas survives so long as this, and its duration in any locality can best be determined by repeated experimental plantings on infested ground during a series of years.

RESISTANT VARIETIES

Observation of the behavior of varieties of peas grown on diseased soil in several places has given rise to the opinion, more or less current among growers, that some varieties of peas show resistance and will produce a better crop than others. Inasmuch as Wilber Brotherton has been studying resistance under field conditions on an extensive scale and will report results in the near future, no attempt will be made here to consider the merits of commercial varieties. An attempt has been made to supplement Brotherton's studies by seeking to devise laboratory methods whereby disease resistance can be tested more rapidly than in field trials, and also to learn in so far as possible the nature of the resistance which varieties may possess.

The attempt to test varieties in the greenhouse under controlled conditions has not yet developed a method that is satisfactory. In the greenhouse in winter there is an apparent obliteration or reduction of differences in behavior compared with that observed in the field not unlike that which occurred in an earlier study (*8*, p. 471) of resistance of peas to a species of Fusarium. This work is still in progress and may be reported more fully later.

It may be said here, however, that no variety of peas, whether of the garden or of the field varieties, has been found immune to the fungus. The hardiest variety of field pea which produces a crop on soil so infested that few plants of garden varieties survive to maturity suffers almost if not quite as great a loss of cortex from its roots as its more susceptible neighbors. In spite of this loss it maintains growth and matures seed. This difference in behavior, termed resistance, seems to be due to several characters which resistant plants possess to a greater degree than those which perish. There is some evidence indicating that the fungus traverses the cortex of some varieties much more slowly than that of others, thus destroying roots less rapidly. Finally, when cortex is destroyed the remaining vascular cylinder of some plants seems to be able to exclude bacteria and fungi in the soil and to function quite efficiently thus denuded of absorbing tissue.

Whatever the importance of these several factors in resistance mentioned above, it will be seen at once that the growth of resistant peas

permits the infestation of the soil with the fungus to proceed as rapidly as when the most susceptible peas are grown. With intensive culture these resistant peas will be subjected to increasingly severe infection from year to year. Resistant varieties, however, may afford a very satisfactory escape from root rot where it is not extremely severe. But it remains to be determined experimentally whether any resistance has yet been found in varieties of commercial utility which will continue to produce profitable crops with intensive culture on soil in which this disease reaches its greatest severity.

SUMMARY

1. Of the several root-rot diseases of peas occurring in the United States which have been distinguished and studied during the past five years, the disease caused by the fungus described in this paper appears to be the most important. It occurs in nearly all of the important pea-growing districts with a varying severity which depends largely upon the degree to which intensive culture of peas permits the accumulation of the fungus in the soil and upon the conditions of soil temperature and moisture favoring early infection and rapid decay of the invaded roots. No other crop than peas has been found subject to disease from this fungus.

2. The effect of this disease upon the appearance of the plant in the field and upon the yield of the crop varies with the stage of development of the plant at which infection takes place and upon the number of infections. If the root system is invaded extensively when only three or four nodes have been formed, the plant may wilt and die suddenly. Later invasions may result in dwarfing of growth with drying out of foliage from the ground upward and in unproductivity. The disease can hardly be distinguished by the appearance of the top of the plant, but it can usually be identified readily when plants are pulled from the ground by the behavior of the root, which instead of breaking near the planted seed, pulls out as a fibrous string consisting of the vascular cylinder of the root freed from the decayed cortex.

3. The fungus enters only the cortex of the roots and base of the stem, where it produces a softening and rapid decay of the tissue, leaving the vascular cylinder exposed to decay by other organisms. In most varieties of garden peas the smaller roots thus denuded of cortex die immediately. A large number of oospores are formed by the fungus in the invaded cortex, and it appears to be from these spores, which increase in the soil from year to year with intensive culture of the crop, that infection originates each season.

4. The fungus can be isolated in pure culture only with considerable difficulty, both because the period during which the mycelium is growing actively in the tissue is brief and because it is so closely associated with bacteria and other fungi that the separation of the parasite from its associates is not readily accomplished. However, 12 cultures from different localities have been obtained for comparison and study.

5. Within the host tissues or on a suitable solid substratum the mycelium soon gives rise to resistant thick-walled bodies, the oospores which result from the development of oogonia following their fertilization by antheridia, of which from one to five are associated with each female cell. Depending on the presence or absence of food materials, the oospores germinate either directly by the production of one to several vegetative hyphae, or indirectly by the proliferation of a single germ hypha of limited growth, within which, as well as within the oospore wall, the protoplasm is divided into portions 13 to 18 in number, which are promptly discharged in the manner characteristic of the genus.

6. Asexual reproduction resulting in the formation of great numbers of motile zoospores ensues whenever actively growing mycelium is provided with suitable conditions. Young thalli may become almost entirely involved in sporogenesis, the individual sporangia being represented by portions of mycelium delimited by septa and often including a moderate number of well-developed branches. These sporangial units discharge their zoospores by one or several elements, the distal portions of which are considerably constricted.

7. The fungus shows much similarity to two aquatic congeners possessing smooth oogonia, *Aphanomyces laevis* and *A. helicoides*, differing especially, however, from the former in having a greatly thickened oogonial envelope with characteristically sinuous internal contour, and from the latter in its antheridial branches not exhibiting any well-defined spiral habit. It is described as a new species, *Aphanomyces euteiches* Drechsler.

8. Inoculation of pea plants with pure cultures under conditions of controlled soil temperature and moisture show that infection of peas may take place at temperatures between 10° and 30° C., but that optimum

temperature for development of the disease is approximately between 15° and 30°. Differences in soil moisture gave little difference in infection under the conditions of these experiments, whereas observation in the field seems to show that the disease is more severe on soil with high moisture-holding capacity or on soil in which water is held by impervious subsoil or by subirrigation.

9. The fungus appears to occur widely in cultivated soils and may be conveyed from infested fields to others by any agency that carries soil. There is no evidence indicating that it is carried with seed.

10. The disease can be prevented and controlled most effectively by crop rotation. The length of rotation required appears to vary greatly with local conditions. On certain irrigated soils which appear to have such low moisture-holding capacity that they provide unfavorable conditions for the development of the fungus, the disease has not appeared or has not become destructive even after peas have been grown nearly every year for 10 years. The disease has become destructive on similar soil when it is subirrigated. On some soils in humid territory the third successive crop of peas is often badly damaged by this root rot, and a comparatively long rotation appears to be necessary to prevent it from accumulating in the soil. In some soils which have become heavily infested the fungus appears to have persisted in sufficient amount to produce conspicuous injury to peas after a six-year rotation.

11. Although all varieties of field and garden peas are subject to this disease, there is considerable difference in the amount of injury which they incur from it, especially in situations where the disease does not develop great severity. Study of the nature and commercial value of this resistance is in progress.

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