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APHANOMYCES SPECIES AND THEIR ROOT DISEASES IN PEA AND SUGARBEET
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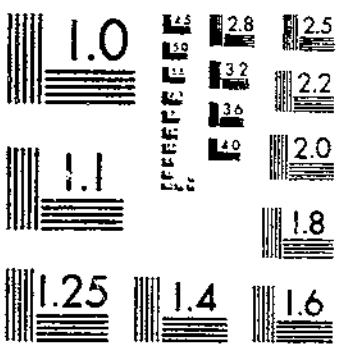

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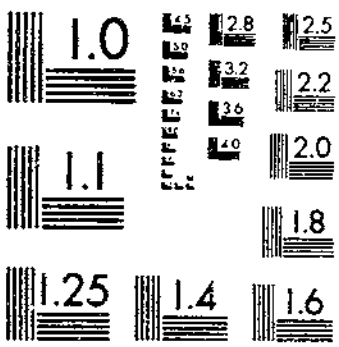

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APHANOMYCES SPECIES AND THEIR ROOT DISEASES IN PEA AND SUGARBEET

A REVIEW

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Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

CONTENTS

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APHANOMYCES SPECIES AND THEIR ROOT DISEASES IN PEA AND SUGARBEET

By GEORGE C. PAPAVIZAS and WILLIAM A. AYERS,
microbiologists, Soilborne Diseases Laboratory,
Northeastern Region, Agricultural Research Service

Root rots of pea (*Pisum sativum* L.) are incited by any one of several different pathogenic fungi or by a combination of fungi. The most common pathogens, listed in order of their economic importance, are *Aphanomyces euteiches* Drechs. (*125–128, 160, 254, 289*),[1] *Fusarium solani* (Mart.) Appel & Wr. f. sp. *pisi* (Jones) Snyd. & Hans., *Pythium ultimum* Trow, *Rhizoctonia solani* Kühn, and *Ascochyta pinodella* Jones (*332*). *A. euteiches* is one of the most destructive pea pathogens in the commercial growing areas of Wisconsin (*161, 313, 348, 349*), Minnesota (*335, 336*), and New York (*281*), and it is of major economic importance in many other pea-growing areas in the United States (*196, 253, 342*) and elsewhere (*114, 232*).

Aphanomyces root rot depends on high soil moisture for its initiation and rapid spread. It is more severe in wet seasons at soil temperatures from 22° to 28° C. and in soils with a high water-retaining capacity. When soils become infested with *A. euteiches*, they may remain potentially dangerous for pea production for several years (*303, 304*). No resistance has been incorporated into commercial pea cultivars and no fungicides are known to control the disease economically in the field. Crop rotations of even 10 years may not always provide effective control of the disease. The only commercial "control" available is the avoidance of fields known to be infested with *A. euteiches*.

Blackroot is one of the most serious diseases of sugarbeet (*Beta vulgaris* L.) in the midcontinental humid belt of the United States and Canada and in other sugarbeet-growing sections of the United States and Europe. Several pathogenic fungi, including *Pythium* spp., *Phoma betae* Frank, *R. solani*, and *Aphanomyces cochlioides* Drechs., have been implicated in causing blackroot and death of seedlings (*45, 46, 50, 105*). *A. cochlioides*, which is the major pathogen in the blackroot complex (*71, 72*), is a limiting factor in

[1] Italic numbers in parentheses refer to Literature Cited, p. 134.

sugarbeet production in the United States (*72, 110*), Ontario (*146, 200*), and Europe (*323–325*). Continual cropping to sugarbeets may increase soil infestation to such an extent that it soon becomes uneconomical to grow sugarbeets.

Of 13 accepted species in the genus *Aphanomyces* (*283*), only *A. euteiches* and *A. cochlioides* can be considered as plant pathogens of great economic importance. These two species have been the subject of extensive investigation for more than 40 years. There are about 500 publications pertaining to them. At least 15 Ph. D. dissertations have been prepared on the two pathogens since 1958. Despite this, there have been no symposia or comprehensive reviews dealing with these two pathogens and the diseases they cause. Thus a review of the accumulated data is long overdue.

This review summarizes and puts into perspective our present knowledge of *A. euteiches* and *A. cochlioides* and the diseases they cause. It also provides a critical analysis of the biological processes that seem relevant to the development of the diseases incited by the two species and to the implementation of control measures. We hope that this summarized information and bibliography will stimulate increased research, which may soon lead to a full understanding of the biology and ecology of the two pathogens and to the development of ecologically acceptable, economic control measures.

HISTORICAL REVIEW

In 1860 deBary (*88*) established a new genus of aquatic fungi, *Aphanomyces*, to include several saprophytic and parasitic fungi observed during this period. He described germination of oospores of *A. stellatus* deBary, the type species of his new genus, which he maintained in water culture for 3 months. Sorokine (*293*) repeated some of deBary's work on *Aphanomyces*. He observed that oospores of *A. stellatus* could germinate not only by germ tubes, the manner described by deBary, but also by producing a short germ tube that protruded out of the oogonial wall and formed zoospores. In Sorokine's experiments germination occurred only if the oospores remained in the light. Subsequent findings by Kasanowsky (*162*) on *A. laevis* deBary closely paralleled those of deBary. Kasanowsky observed germinating oospores of *A. laevis* for the first time. His drawings, however, indicated that he was most probably dealing with a type more nearly resembling the Swiss form of *A. laevis* described as *A. helicoides* by Minden (*213*) than the one originally described by deBary.

Peters (*245*) reported the outbreak of a disease called "Wurzelbrand," which severely affected sugarbeets throughout Germany.

According to his account, the disease was caused by *Pythium debaryanum* Hesse, *Phoma betae*, and a species of *Aphanomyces*. Because of the absence of protuberances from the oogonial wall and a certain degree of similarity in the appearance and size of the female organs to those of *A. laevis*, the parasite causing "Wurzelbrand," together with *P. debaryanum* and *P. betae*, was identified as *A. laevis*. Later in a more complete account of the three sugarbeet pathogens, Peters (*246*) continued to regard the form of *Aphanomyces* from sugarbeets as identical with *A. laevis* despite certain differences from deBary's (*88*) original description of *A. laevis*. Barrett (*33*) reported *A. laevis* as the cause of blackroot rot of radishes that had been observed frequently throughout the United States. Later Kendrick (*163*) named the radish water mold *A. raphani* Kendr.

In 1913 Edson (*104*) reported that *A. laevis* was involved in the damping-off and root rot complex of sugarbeets in the United States. Later he (*106*) observed that the American fungus was not identical with the European beet parasite. His water mold differed morphologically from deBary's *A. laevis* in the method of zoospore formation, and he assigned it to a new genus and species, which he named *Rheosporangium aphanidermatum* Edson. The new genus was subsequently placed in the genus *Pythium* (*111*) and the sugarbeet blackroot pathogen was referred to as *P. aphanidermatum* (Edson) Fitz. With the assignment of the water mold to the genus *Pythium*, reference to *A. laevis* as an active cause of blackroot of sugarbeets in the United States came to an end. As a result of the early taxonomic work on *A. laevis* and its early association with "Wurzelbrand" of sugarbeets by Peters (*245*), the binomial *A. laevis* has been cited rather frequently in the European literature among the names of parasitic fungi responsible for blackroot.

Drechsler (*100*) named and described *A. cochlioides* Drechs., which he originally isolated from diseased sugarbeet seedlings in Michigan. In a more complete account he (*101*) demonstrated the pathogenicity of *A. cochlioides* on sugarbeet seedlings and stated that his *A. cochlioides* was probably identical with the water mold causing "Wurzelbrand" of sugarbeets in Germany and other European countries.

A. euteiches Drechs. was first reported by Drechsler (*99*) and described by Jones and Drechsler (*160*) as a new parasite inciting root rot of peas. Jones and Drechsler observed that their new pathogen invaded the root cortex and the basal stem of peas and developed thick-walled oospores in the rotted tissue. They considered the oospores to be the stage of the pathogen responsible

for prolonged survival in soil. They reported that common root rot of peas had been present in the United States for a long period prior to 1925. They also stated that the disease occurs in all pea-growing regions of the country and that it is more important than all other diseases of peas combined.

Root rots of peas and sugarbeets began to attract serious attention very soon after the inception of the pea-canning industry and about the time Drechsler described *A. euteiches* and *A. cochlioides*. In 1924 Coons (*66*) observed that the preceding crop affected the incidence of blackroot of sugarbeets. In 1924 Jones and Linford (*161*) performed an elaborate and extensive survey to determine the importance of various pea diseases in Wisconsin and published one of the first classical papers on *A. euteiches* in 1925. They considered *Aphanomyces* root rot as the most important disease of peas in Wisconsin.

From 1889, when the first canning factories were established in Wisconsin, until about 1912, almost all canning companies owned land for pea cultivation. As many as 10 successive pea crops were grown on company-owned land in many instances. The Wisconsin Agricultural Experiment Station became interested in the disease problems of the canning industry where pea crops began to fail after 1910 as a result of this rather intensive pea monoculture, especially in wet locations. It did not take long for scientists to see the effect of early land management policies on the pea-canning industry and to conclude that peas could not be grown successfully when they were continuously cropped on the same fields.

By 1915, on the advice of the experiment station scientists, the companies began growing less acreage of peas on company-owned land and increased the number of farmers under contract to grow peas for the companies. With more contract farming, pea cultivation was dispersed, resulting in less disastrous and less frequent crop failures. This management policy change allowed the pea-canning industry in Wisconsin to expand to such an extent that by 1924 canning peas were grown on 102,000 acres, with 135 plants processing the yield, which was worth $7 million.

Research on *A. euteiches* has continued to increase since 1925. In the late 1940's and early 1950's Minnesota (*335, 342*), Wisconsin (*285, 348*), and New York (*280, 282*) and several State institutions and private companies undertook an extensive program to study root rot epidemiology and survival of the pea water mold and to develop pea lines resistant to disease and other control measures for *A. euteiches*.

In addition to the early classical work of Drechsler, the U.S. Department of Agriculture also undertook an extensive research

program on the physiology and control of *A. euteiches* that resulted in several publications (*85, 86, 234, 235, 238, 239*). Investigations also intensified in number and scope on *A. cochlioides* in various States and by the U.S. Department of Agriculture, which pioneered in the development of various sugarbeet strains tolerant or resistant to *A. cochlioides* (*41, 42, 63, 64, 68, 74–76, 112, 269, 272, 274*).

GEOGRAPHIC DISTRIBUTION

A. euteiches occurs in North America, Europe, and Australia. In the United States it was found in practically every pea-growing district that was thoroughly investigated. It occurs frequently, and often destructively, in the Eastern and Central States (fig. 1, *A*).

In the United States it is found in Wisconsin (*90, 161, 313*), Minnesota (*294, 335*), and Michigan (*188, 196*); in New Jersey, Delaware, Maryland, and Virginia (*99, 125, 126*), where it was thought to be the most important primary cause of pea root rot; in Connecticut (*111*) and New York (*254, 255*); in Utah, Idaho, and Montana, where it appears to be unimportant (*160, 165, 258*); in the Pacific Northwest, where it seldom appears to be severe except in irrigated land (*160*); in the Southeastern United States (*116, 259, 317, 318*); and in Ontario, Canada (*201*).

A. euteiches appears to be very important in the States bordering the Great Lakes, where soil moisture is high during the spring and summer and where most of the peas are grown in the United States. In New York, for instance, *A. euteiches* was found in six of 10 counties examined in 1937 and in nine of 10 examined in 1940 (*254*). On the other hand, it was isolated infrequently and appeared to be unimportant in Ontario and the Western United States.

In northern Europe *A. euteiches* was discovered in the Seine-et-Oise district of France, where it caused heavy losses in 1932–33 (*174, 175*); in England and Wales (*34, 123, 249*); in Norway (*299, 300*), southern Sweden, where it caused great economic losses regularly (*232*), and Denmark (*122, 290–292*); and in the nonchernozem zone of the U.S.S.R. (*172, 173*) (fig. 1, *B*). It was found in Jamaica (*288*), Australia (*311*), and Tasmania (*4, 114, 298*).

Reports from Tasmania on the importance of *A. euteiches* are contradictory. Geach (*114*) observed great losses on gray peas in Tasmania incited by *A. euteiches*. The average pea yield for 1933–34 was 18.4 bushels per acre. Because of *A. euteiches*, the yield was reduced to about 10 bushels per acre or less, and in some

areas only the amount of seed sown was harvested (*114*). Later the impression was given by Geard (*115*) that *A. euteiches* ceased to be troublesome in the Longford-Cressy area of Tasmania because the growers learned to avoid poorly drained fields. This view

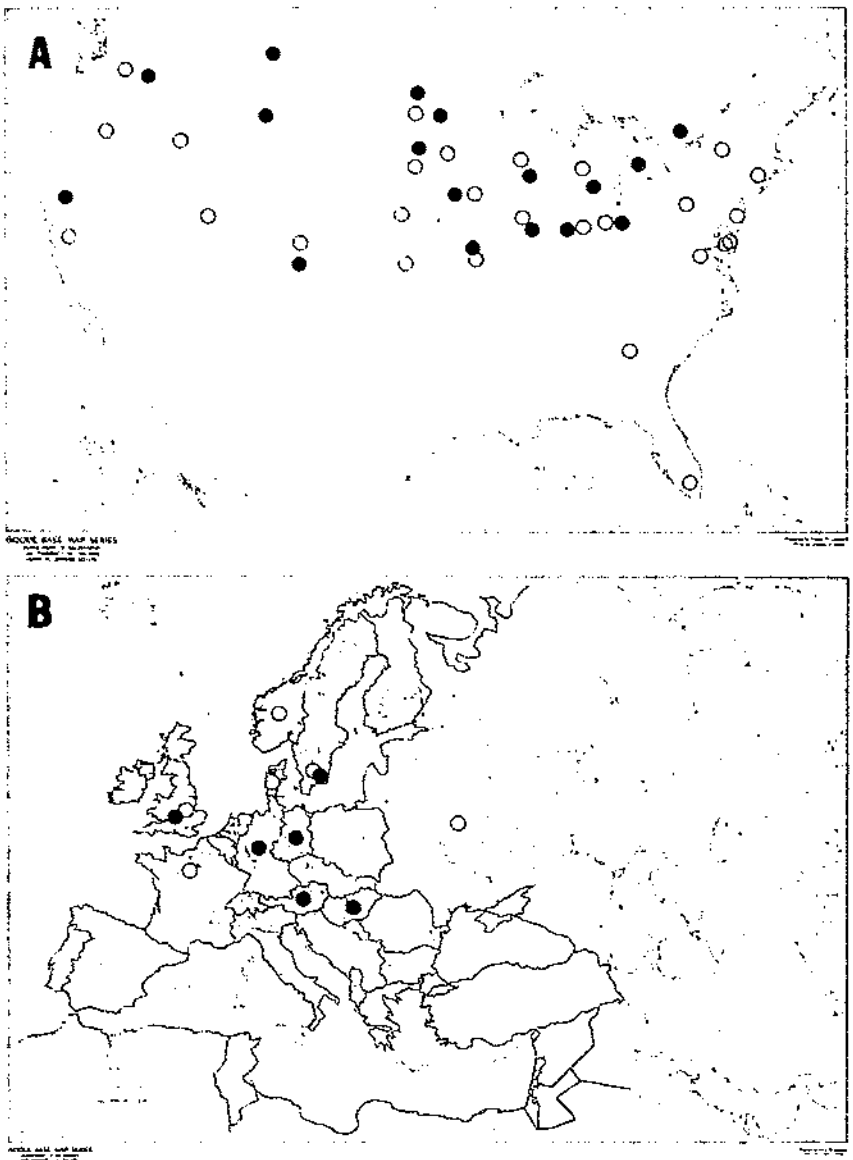


FIGURE 1.—Distribution of *Aphanomyces euteiches* (open circles) and *A. cochlioides* (solid circles) based on the literature: *A*, United States and Canada; *B*, Europe.

was not shared by Stubbs (*298*), who found that *A. euteiches*, together with other root pathogens, forced abandonment of pea production in some areas in Tasmania.

A. cochlioides occurs in America and Europe (fig. 1). It is a pathogen of major importance in Minnesota and Iowa (*46, 209, 306, 339, 347*), Ohio (*72, 316*), the Dakotas (*46, 334*), Montana (*9, 10, 18, 220*), and Washington (*56*). In the southwestern part of the Province of Ontario *A. cochlioides* causes serious losses on sugarbeets (*149, 200*). Other, but less frequent, observations of the fungus in the United States have been reported in California (*152, 176*) and in southern Alberta, Canada (*78*). In Germany *A. cochlioides* has been a serious pathogen of sugarbeets for many decades (*1–3, 245, 247, 323*). According to the German Biological Institute at Dahlem (*247*), the blackroot pathogen was responsible for 11 percent of the total cases of root rot of beets from 1906 to 1908. Sporadic reports are found in the literature for the occurrence of *A. cochlioides* in Austria (*211*), England (*248*), Sweden (*27*), Hungary (*124*), and Denmark (*118*). The "caida" disease of sugarbeets incited by *A. cochlioides* is considered responsible for considerable losses of young plants in the fields of Chile (*295, 322, 323*).

DISTRIBUTION IN RELATION TO SOIL DEPTH AND TYPE

Reports have been only sporadic on the distribution of *A. euteiches* and *A. cochlioides* in relation to soil depth. *A. cochlioides* was found to be most abundant in the top 2 inches of Brookston clay loam soil in Ontario (*200*). The pathogen could be detected down to 6 inches but not at 8 inches. Burke et al. (*52*) determined *A. euteiches* populations at various depths in "root rot" and "non-root rot" soils in southern Wisconsin by growing pea seedlings in soil samples and determining the disease severity indexes. More *A. euteiches* pathogen was detected in the plowed layer than in subsoils in nine of 12 pea "root rot" fields and in two of 12 "non-root rot" fields. From these limited observations it appears that *A. euteiches* and *A. cochlioides* are found primarily in the plowed layer of soil (0–6 inches) in infested fields.

In regard to soil type, *A. cochlioides* was isolated in great abundance by McKeen (*200*) from clay soils in Ontario, Canada. On the contrary, *A. euteiches* was isolated in New York by Reinking (*254*) from plants growing in a variety of soils differing in reaction (pH 5.4–7.5) and texture. In 1940 *A. euteiches* was found by Reinking in Ontario, Fox gravely, silty clay (bottom land and

upland), and silt loams. It was not found in soils that never had peas before, but it was abundant in silty clay loam previously cropped to peas near Geneva, N.Y. Earlier data on the soil type favoring *A. euteiches* (*161*, *313*, *315*) are somewhat at variance with those of Reinking. According to these investigators, development of *A. euteiches* root rot of peas was favored more in heavy clay soils than in light soils.

Walker and Hare (*313*) showed that there was some relationship of soil type to *A. euteiches* root rot severity. The Disease Severity Index (DSI) was higher in peas grown in clay soils than in other soils; however, they could not detect a direct correlation of DSI and water-holding capacity (WHC) of the soil type, since loams and prairie soils are expected to have higher WHC than sandy loams. No explanation was offered by Walker and Hare as to why peas in sandy soils had the same DSI as those in loam soils. It is highly probable that the soils used by Walker and Hare had different inoculum potential of *A. euteiches* to begin with. More research is needed to clarify the interrelationships among soil texture and WHC, inoculum density, and disease potential of *A. euteiches*.

ECONOMIC IMPORTANCE

Estimation of crop losses caused by *A. euteiches* and *A. cochlioides* from yield figures is extremely difficult since numerous other factors may be responsible for variation in yield. Because of the almost universal association of *Aphanomyces* with other parasitic and quasi-parasitic fungi, it is even more difficult to ascertain the precise proportion of economic losses due to *Aphanomyces* and to estimate its exact economic importance. Unless a plant is dead or dying from *Aphanomyces*, it often is not reported in disease surveys or its condition may be attributed to other causes.

Aphanomyces euteiches

Zaumeyer (*332*) stated concerning pea diseases that "taking the country as a whole, more loss is believed to be caused by root rots than by any other single disease and possibly more than by all other diseases combined." *A. euteiches* was considered by Zaumeyer as one of the most important pathogens in the pea root rot complex. The common root rot of peas incited by *A. euteiches* is considered a limiting factor in pea production in the Midwest and elsewhere in the United States (*313*, *335*). In Minnesota, for instance, it is estimated conservatively that *A. euteiches* may account for 80 percent of the root rots of peas (*342*).

A. euteiches is also of major economic importance in other areas of the world. Common root rot was of major importance on gray peas in the Longford-Cressy area of Tasmania in the 1920's and 1930's (*114*), in some pea-growing regions of the nonchernozem zone of the U.S.S.R. (*172, 173*), and in Sweden (*232*). During some years when the temperature and moisture are favorable for *A. euteiches* development the damage can be so important in Sweden that the crop is not harvested.

A. euteiches can be found in almost every field where peas can be grown. In the middle 1920's Jones and Drechsler (*160*) believed that as much as 25 percent of the total acreage of peas in the Eastern and Central United States was infested with *A. euteiches*. Jones and Linford (*161*) also estimated that thousands of acres were severely damaged by this pathogen each year.

The results of the disease survey, conducted by Jones and Linford in which 688 fields comprising 5,416 acres were examined, showed that root rot was present in 32 percent of the fields and 11 percent were severely infested. Of the fields examined, 48 percent had their first crop of peas and root rot was rarely encountered. However, of the fields where the fifth crop of peas was growing, 56 percent were severely infested. The total loss in inspected fields was estimated by Jones and Linford at 8 percent of the total yield. As a result of this survey, they concluded that the root rot pathogen was more destructive than all other fungal and bacterial diseases of peas combined. Jones and Drechsler (*160*) also concluded that *A. euteiches* was destructive in every pea-growing area of the United States, rendering many thousands of acres unprofitable or destroyed every year.

Smith and Walker (*289*) estimated that root rot due to *A. euteiches* may cause a reduction in yield equivalent to 10 percent of the total crop in a year with favorable environmental conditions for root rot development in Wisconsin. In another pea disease survey in Wisconsin, Walker and Hare (*313*) compared pea yields in fields showing no disease or slight disease with yields in fields showing moderate to severe root rot. The yield in the latter fields varied from 8 to 34 percent less than that in fields with zero to slight root rot. They estimated that the average loss from *A. euteiches* root rot in moderately or severely affected fields was 25 to 30 percent.

The U.S. Department of Agriculture (*308*) estimated that root rot of green peas caused by several soilborne fungi, primarily *A. euteiches*, resulted in a 10-percent average annual loss during 1951–60 in the United States. This represents an annual loss of about 1,142,080 hundredweight or $5.2 million. This figure is an

underestimate of actual losses because it includes losses only on peas for the fresh market and processing but not on dry peas. In addition to these direct losses in quantity, infections by *A. euteiches* may also result in inferior pea quality, uneven maturation, and prolonged abandonment of peafields.

Aphanomyces cochlioides

A. cochlioides more than any other factor limits production of sugarbeets in many areas of the United States. The low sugarbeet yields in the humid area of the United States in the past can definitely be attributed to poor stands as a result of blackroot and to the continuous deteriorating effect of the chronic phase of *A. cochlioides* on the crop (*73, 109, 110, 347*). Great economic losses are caused by *A. cochlioides* on sugarbeets in Western Germany (*296, 297, 323*), central Chile (*323*), and Canada (*149, 200*). In the North Central States and in Canada it is not uncommon for a large acreage of sugarbeets to be abandoned in the spring because of blackroot, which results in poor, gappy stands. This condition not only results in serious economic losses but also upsets the crop rotation schedules.

During the most serious phase of damping-off, the postemergence phase, stands may be either completely destroyed or reduced to an extent that it becomes questionable whether they should be saved, even though following the period of high mortality, the surviving seedlings show evidence of "coming out" of blackroot (*149*). Since field stands below 80 percent of full stand are not compensated by the larger remaining plants, the damping-off phase results in significant loss of yield. Abandonment of fields because of blackroot may have a consequent depressing effect on future acreage planted to sugarbeets. Despite the high cash value anticipated from an acre of sugarbeets, the grower may hesitate to plant sugarbeets following a serious outbreak of blackroot when faced with the possibility of another partial or total crop loss. Sugar factories then cannot contract sufficient acreage for profitable operations within reasonable economic shipping distances. Factories must depend on scattered, reduced acreage and obtain beets from longer distances at higher cost.

Reduction in sugarbeet stands may also affect mechanization processes on which the industry depends so much. Mechanical thinning may not be applied safely to fields in which the drill rows show extensive plant gaps. In addition to stand reductions during the acute blackroot phase and all the undesirable secondary effects of this reduction, many of the remaining plants will continue to

suffer from the chronic phase. The damping-off problem thus expands and extends into a chronic root disease problem in the remaining stand until harvest. The result is abandonment of acreage or expensive replanting during a busy season.

In a survey of 117 sugarbeet fields in western Washington conducted by Campbell (*56*) during the summer of 1937, blackroot ranged from a fraction of 1 percent to 95 percent of the fields. Certain fields showed no losses. Others were so severely damaged by the disease that the farmers had to abandon them. A study made by Lill (*184*) showed for a typical sugarbeet district in the United States that the average stand of beets over a 5-year period ranged from 63 to 69 percent, so low that root yields could not reach half the normal production. Studies conducted by Coons et al. (*72*) showed that in the blackroot complex *A. cochlioides* was the most important organism in causing greatly reduced yield. *Pythium* spp., *Phoma betae*, and *Rhizoctonia solani* were amenable to control, at least to some extent, by seed treatments with fungicides. In contrast, the chronic disease caused by *A. cochlioides* could not be controlled.

Although there are few estimates of the actual losses in sugarbeets, Schneider (*347*) reported that on the average more than 10-percent reduction occurred in sugar production each year in the humid areas of the United States. Stands may have been reduced as much as 30 to 40 percent by *A. cochlioides* in most of the factory districts where blackroot occurred. The U.S. Department of Agriculture (*308*) estimated very conservatively that blackroot alone caused an average annual loss of 1 percent over a 10-year period. In the United States this represents an average loss of approximately 200,000 tons of sugarbeets worth $2.4 million. This does not include losses incurred by the chronic phase of *A. cochlioides*. If one assumes that *A. cochlioides* reduces yields of sugarbeets throughout the world by an average of 1 percent, then the 1964 world loss equaled 2.2 million tons of sugarbeets (*309*). If the value of sugarbeets was $12 per ton in 1964, the total world loss of sugarbeets due to the acute phase of *A. cochlioides* was approximately $26.5 million.

HOST RANGE

Several lists of plants that may be parasitized by *A. euteiches* or *A. cochlioides* can be found in the literature. With few exceptions, these lists contain an incomplete and limited amount of information and they should therefore be utilized or interpreted

with caution. Their deficiencies usually stem from the following facts:

(1) Although some of the hosts were evaluated by observing plants grown in field soil infested with *Aphanomyces*, most of the hosts were evaluated by growing plants in sterilized media inoculated with known isolates of the pathogen. It is possible that plants tested as hosts of *Aphanomyces* in media free of other micro-organisms, where antagonism to the pathogen is practically nonexistent, would be less susceptible to the pathogen in natural soil in the greenhouse and least susceptible in the field.

(2) With few exceptions, there was no attempt to standardize the kind and concentration of inoculum used or to use more than one isolate of the pathogen.

(3) Most investigators employed only one cultivar of the host plants to be tested. Had they used different cultivars, different kinds of plants, or even different sets of environmental conditions, results on the host range might have been different.

When a plant species is reported more than once in the literature as a host of *A. euteiches* or *A. cochlioides*, the year the reference was published indicates who reported the plant first as a host. If an artificial inoculum was used, the investigator who performed the experiment was listed as the authority for the report. Incomplete host lists were also used, but the proper host binomials, common names, and authorities were supplied from other sources.[2] Because of variations in the nomenclature of the hosts of *Aphanomyces* through the years, it is extremely difficult to cite with certainty the total number of species parasitized by *A. euteiches* and *A. cochlioides*.

Aphanomyces euteiches

It is difficult to establish a working concept of the meaning of "host range" in *A. euteiches*. Consequently, the host list of this pathogen (table 1) represents at best only an approximation of the host range of this fungus. Unfortunately, with few exceptions, most of the parasitization studies of plants other than peas have been performed by pure culture inoculations. Haenseler (*126*) and

[2] The following references were consulted for supplying host binomials and common names not supplied by the original papers or for possible synonymy: (1) Bailey, L. H., "Manual of Cultivated Plants," rev. ed., 1116 pp., illus., The MacMillan Co., London and New York, 1949. (2) Bailey, L. H., "The Standard Cyclopedia of Horticulture," 3639 pp., illus., The MacMillan Co., London and New York, 1930. (3) Seymour, A. B., "Host Index of the Fungi of North America," 732 pp., Harvard Univ. Press, Cambridge, 1929.

Linford (*185*) tested the susceptibility of many hosts to *A. euteiches* by planting the species in naturally infested soil. Few subsequent investigators (*102, 114, 145*) used nonsterile soil for limited host range studies. The new and more numerous systematic host range studies in table 1 are based on pure culture inoculations employing entire plants (*59, 107, 287, 335*) or excised root tips of plants (*335, 336*). Although these studies in sterile media provide an extensive host list, the question still remains whether all these plants may actually be attacked by *A. euteiches* in the field.

TABLE 1.—*Plant species reported as hosts of* Aphanomyces euteiches

Host	Common name	Reference
ALISMATACEAE		
Echinodorus brevipedicellatus Buch	Amazon sword plant	Ridings and Zettler (*259*).
AMARANTHACEAE		
Amaranthus retroflexus L	Red root pigweed	Sherwood and Hagedorn (*287*), Carlson (*336*).
CARYOPHYLLACEAE		
Lychnis alba Mill	White cockle	Carlson (*336*).
CHENOPODIACEAE		
Beta vulgaris L	Garden beet	Do.
Chenopodium album L	Lambsquarters	Do.
Kochia scoparia (L.) Schrad	Fireweed	Do.
Spinacia oleracea L	Spinach	Sherwood and Hagedorn (*287*), Carlson (*336*).
COMPOSITAE		
Eupatorium rugosum Houtt	White snakeroot	Carlson (*336*).
Helianthus annuus L	Sunflower	Do.
Lactuca sativa L.[1]	Lettuce	Mix (*219*).
L. sativa L	do	Carlson (*336*).
Sonchus arvensis L	Sowthistle	Do.
CRUCIFERAE		
Brassica oleracea var. *capitata* L	Cabbage	Do.
Lepidium sativum L	Peppergrass	Do.
Raphanus sativus L	Radish	Do.
CUCURBITACEAE		
Cucumis sativus L	Cucumber	Do.

See footnote at end of table.

TABLE 1.—*Plant species reported as hosts of* Aphanomyces euteiches—Continued

Host	Common name	Reference
GRAMINEAE		
Avena sativa L.[1]	Oat	Geach (*114*).
A. sativa L	do	Carley (*335*), Carlson (*336*).
Dactylis glomerata L	Orchardgrass	Carlson (*336*).
Hordeum vulgare L	Barley	Do.
Panicum miliaceum L	Proso	Do.
Phalaris arundinacea L.	Reed canarygrass	Do.
Setaria italica (L.) Beauv	German foxtail millet	Do.
Sorghum bicolor (L.) Moench	Sorghum, Waconia sorghum	Do.
Triticum aestivum L	Wheat	Carley (*335*), Carlson (*336*).
Zea mays L	Corn	Haenseler (*126*), Carley (*335*).
Z. mays var. *saccharata* (Sturtev.) Bailey	Sweet corn	Carlson (*336*).
LEGUMINOSAE		
Astragalus cicer L	Milkvetch	Sherwood and Hagedorn (*287*).
Cicer arietinum L	Chickpea	Do.
Crotalaria spectabilis Roth	Snowy crotalaria	Do.
Cyamopsis tetragonoloba (L.) Taub	Guar	Do.
Glycine max (L.) Merr	Soybean	Haenseler (*126*), Carley (*335*), Carlson (*336*).
Lathyrus sp		Linford (*185*).
L. cicera L	Flatpod pea	Sherwood and Hagedorn (*287*).
L. hirsutus L	Rough pea	Do.
L. latifolius L	Perennial pea	Linford (*185*).
L. odoratus L.[1]	Sweetpea	Geach (*114*), Linford (*185*).
L. odoratus L	do	Sherwood and Hagedorn (*287*).
L. tingitanus L	Tangier pea	Do.

See footnote at end of table.

TABLE 1.—*Plant species reported as hosts of* Aphanomyces euteiches—Continued

Host	Common name	Reference
LEGUMINOSAE—Con.		
Lotus corniculatus L	Birdsfoot trefoil	Sherwood and Hagedorn (*287*), Carlson (*336*).
Lupinus luteus L	Yellow lupine	Sherwood and Hagedorn (*287*).
Medicago lupulina L	Black medic	Do.
M. orbicularis (L.) All	Button medic	Do.
M. polymorpha L	Burclover	Do.
M. sativa L.[1]	Alfalfa	Linford (*185*).
M. sativa L	do	Haenseler (*126*), Schmitthenner (*262*), Sherwood and Hagedorn (*287*), Carlson (*336*).
Melilotus alba Desr.[1]	White sweetclover	Geach (*114*), Linford, (*185*).
M. alba Desr	do	Haenseler (*126*), Sherwood and Hagedorn (*287*), Carlson (*336*).
M. officinalis (L.) Lam	Yellow sweetclover	Sherwood and Hagedorn (*287*), Carlson (*336*).
Onobrychia viciifolia Scop	Sainfain	Sherwood and Hagedorn (*287*).
Ornithopus sativus Brot	Common serradilla	Do.
Phaseolus aureus Roxb	Mung bean	Sherwood and Hagedorn (*287*), Carlson (*336*).
P. lunatus L	Lima bean	Sherwood and Hagedorn (*287*).

See footnote at end of table.

TABLE 1.—*Plant species reported as hosts of* Aphanomyces euteiches—Continued

Host	Common name	Reference
LEGUMINOSAE—Con.		
P. vulgaris L	Bean	Haenseler (*126*), Sherwood and Hagedorn (*287*), Carley (*335*), Carlson (*336*).
Pisum sativum L	Garden pea	Sherwood and Hagedorn (*287*), Carlson (*336*).
P. sativum subsp. *arvense* (L.) Poir	Field pea	Sherwood and Hagedorn (*287*).
Trifolium hybridum L	Alsike clover	Haenseler (*126*), Sherwood and Hagedorn (*287*), Carlson (*336*).
T. incarnatum L	Crimson clover	Do.
T. pratense L	Red clover	Sherwood and Hagedorn (*287*), Carlson (*336*).
T. repens L	White clover	Do.
T. subterraneum L.[1]	Subterranean clover	Geach (*114*).
Vicia angustifolia L.[1]	Narrowleaf vetch	Geach (*114*), Linford (*185*).
V. benghalensis L	Purple vetch	Geach (*114*).
V. dasycarpa Ten.[1]	Wollypod vetch	Linford (*185*).
V. dasycarpa Ten	do	Sherwood and Hagedorn (*287*).
V. ervilia (L.) Willd.[1]	Bitter vetch	Geach (*114*).
V. ervilia (L.) Willd	do	Linford (*185*).
V. faba L	Broadbean	Carlson (*336*).
V. fulgens Batt.[1]	Scarlet vetch	Linford (*185*).
V. gigantea Hook.[1]		Do.
V. monantha Retz.[1]		Geach (*114*), Linford (*185*).

See footnote at end of table.

TABLE 1.—*Plant species reported as hosts of* Aphanomyces euteiches—Continued

Host	Common name	Reference
LEGUMINOSAE—Con.		
V. pannonica Crantz [1]	Hungarian vetch	Linford (*185*).
V. pannonica Crantz	do	Sherwood and Hagedorn (*287*).
V. sativa L.[1]	Common vetch	Linford (*185*).
V. sativa L	do	Sherwood and Hagedorn (*287*).
V. angustifolia (L.) Walp	Cowpea	Haenseler (*126*), Sherwood and Hagedorn (*287*).
V. villosa Roth	Hairy vetch	Haenseler (*126*), Carlson (*336*).
LILIACEAE		
Allium cepa L	Onion	Carlson (*336*).
LINACEAE		
Linum usitatissimum L	Flax	Sherwood and Hagedorn (*287*), Carlson (*336*).
MALVACEAE		
Malva rotundifolia L	Round leaved mallow	Carlson (*336*).
PINACEAE		
Picea engelmannii Parry ex Engelm	Engelmann spruce	Eliason (*107*).
Pinus banksiana Lamb	Jack pine	Do.
Pseudotsuga menziesii (Mirb.) Franco	Douglas-fir	Do.
POLYGONACEAE		
Rumex acetosa L	Garden sorrel	Carlson (*336*).
PORTULACACEAE		
Portulaca oleracea L	Common purslane	Do.
SCROPHULARIACEAE		
Verbascum thapsus L	Common mullein	Do.
SOLANACEAE		
Lycopersicum esculentum Mill.[1]	Tomato	McKeen (*199*).
L. esculentum Mill	do	Carlson (*336*).

See footnote at end of table.

TABLE 1.—*Plant species reported as hosts of* Aphanomyces euteiches—Continued

Host	Common name	Reference
SOLANACEAE—Con.		
Capsicum frutescens L.[1]	Pepper	Mix (*219*).
Solanum melongena L.[1]	Eggplant	Do.
UMBELLIFERAE		
Apium graveolens L. var. *dulce* (Mill.) Pers.[1]	Celery	Doran et al. (*92*).
Daucus carota L	Carrot	Carlson (*336*).
VIOLACEAE		
Viola cornuta L	Pansy	Meurs (*210*).
V. tricolor L	do	Meurs (*210*), Carlson (*336*).

[1] Infection as determined by pathogenicity tests in nonsterile, naturally infested soil. In all other hosts, infection determined from artificial inoculation.

Carley's data (*335*) suggested that the host list of *A. euteiches* (table 1) would be reduced if the plant species were grown in natural soil infested with the pathogen. His root tip inoculation experiments in the laboratory indicated that beans, corn, oats, soybeans, and wheat were susceptible to *A. euteiches*. His greenhouse studies with the use of a mixed vermiculite culture containing four isolates of *A. euteiches* showed that beans were susceptible, corn slightly susceptible, oats and wheat weakly parasitized, and soybeans and tomatoes immune. In field studies Carley (*335*) was able to isolate the pathogen from beans and peas only.

A considerable amount of contradictory information exists in the literature as to whether certain plant species are hosts of *A. euteiches*. For instance, Drechsler (*99*) reported that tomatoes were susceptible to *A. euteiches*, but Carley (*335*) could not verify it. Sherwood and Hagedorn (*287*) reported that barley, corn, lettuce, oats, onion, pansy, rye, soybeans, and other plants were immune to *A. euteiches*, whereas Carlson (*336*) and others (*210, 335*) found some of them to be susceptible to some extent. Several other similar examples could be cited.

Aphanomyces cochlioides

Drechsler (*100, 101*) was the first to demonstrate the pathogenicity of *A. cochlioides* on sugarbeets. Buchholtz (*46*) and

McKeen (*200*) showed that *Amaranthus retroflexus* (pigweed) and *Chenopodium album* (lambsquarters) belonging to the families Amaranthaceae and Chenopodiaceae, respectively, were also hosts of *A. cochlioides* under sterile conditions (table 2).

TABLE 2.—*Plant species reported as hosts of* Aphanomyces cochlioides

Host	Common name	Reference
AIZOACEAE		
Mollugo verticillata L	Carpetweed	Schneider (*273*).
Tetragonia tetragonioides (Pall.) Ktge	New Zealand spinach	Do.
AMARANTHACEAE		
Amaranthus blitoides Wats	Prostrate pigweed	Do.
A. retroflexus L	Pigweed	Buchholtz (*46*), McKeen (*200*), Schneider (*273*).
Celosia argentea L	Cockscomb	Schneider (*273*).
Gomphrena globosa L	Globe amaranth	Do.
CARYOPHYLLACEAE		
Cerastium sp.[1]	Mouse-ear chickweed	Do.
Dianthus chinensis L	Rainbow pink	Do.
Lychnis alba Mill	White cockle	Do.
L. chalcedonica L.[1]	Maltese cross	Do.
Saponaria ocymoides L	Bouncing-bet	Do.
S. officinalis L.[1]	Bouncing-bet, soapwort	Do.
CHENOPODIACEAE		
Beta lomatogona Fisch. & Mey		Do.
B. patellaris Moq		Do.
B. patula Ait		Do.
B. trigyna Waldsto & Kit		Do.
B. vulgaris L	Sugarbeet	Buchholtz (*46*), Drechsler (*101*), McKeen (*200*), Schneider (*273*).
	Table beet	Schneider (*273*), Downie (*339*).
	Mangel	Schneider (*273*).
B. vulgaris var. *cicla* L	Chard	Do.
Chenopodium album L	Lambsquarters	Buchholtz (*46*), McKeen (*200*), Schneider (*273*).

[1] See footnote at end of table.

TABLE 2.—*Plant species reported as hosts of* Aphanomyces cochlioides—Continued

Host	Common name	Reference
CHENOPODIACEAE—Con.		
Kochia scoparia (L.) Schrad.	Fireweed	Schneider (*278*).
K. scoparia var. *culta* Farwell	Mexican burning bush	Do.
Salsola kali L.	Russian-thistle	Do.
Spinacia oleracea L.	Spinach	Do.
HYDROPHYLLACEAE		
Phacelia campanularia Gray [1]		Do.
LINACEAE		
Linum usitatissimum L.[1]	Flax	Do.
PAPAVERACEAE		
Escholtzia californica Cham	California poppy	Do.
Papaver rhoeas L.	Corn poppy	Do.
PORTULACACEAE		
Portulaca grandiflora Hook.[1]	Moss rose	Do.
P. oleracea L.	Purslane	Do.
SOLANACEAE		
Capsicum frutescens L.[1]	Pepper	McKeen (*199*), Schneider (*278*).

[1] Infection as determined by pathogenicity tests under sterile conditions. In all other hosts, infection confirmed in naturally infested soil.

The most comprehensive studies on the host range of *A. cochlioides* were made over several years by Schneider (*266, 273, 347*). He (*273*) studied seedling reaction of 98 plant species representing 40 families in glass vessels in the laboratory, in artificially infested autoclaved soil in the greenhouse, and in naturally infested soil. Seedlings of 30 species in the following families became infected by zoospores of *A. cochlioides* in the greenhouse: Aizoaceae, Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Hydrophyllaceae, Linaceae, Papaveraceae, Portulacaceae, and Solanaceae (fig. 2).

Pure cultures of *A. cochlioides* were isolated from *Chenopodium album*, *Spinacia oleracea*, *Tetragonia tetragonioides*, *Mollugo verticillata*, and *Saponaria ocymoides* (all grown in naturally infested soil) and were pathogenic to sugarbeets. Schneider (*347*) also found that cultivated forms of *Beta vulgaris* such as table

FN-3678

FIGURE 2.—Plant species inoculated (below) with *Aphanomyces cochlioides*: (1) *Beta vulgaris* (sugarbeet), (2) *Chenopodium album* (lambsquarters), (3) *Amaranthus retroflexus* (pigweed), (4) *Cerastium* sp. (chickweed). Plants not inoculated (above). (Courtesy of C. L. Schneider.)

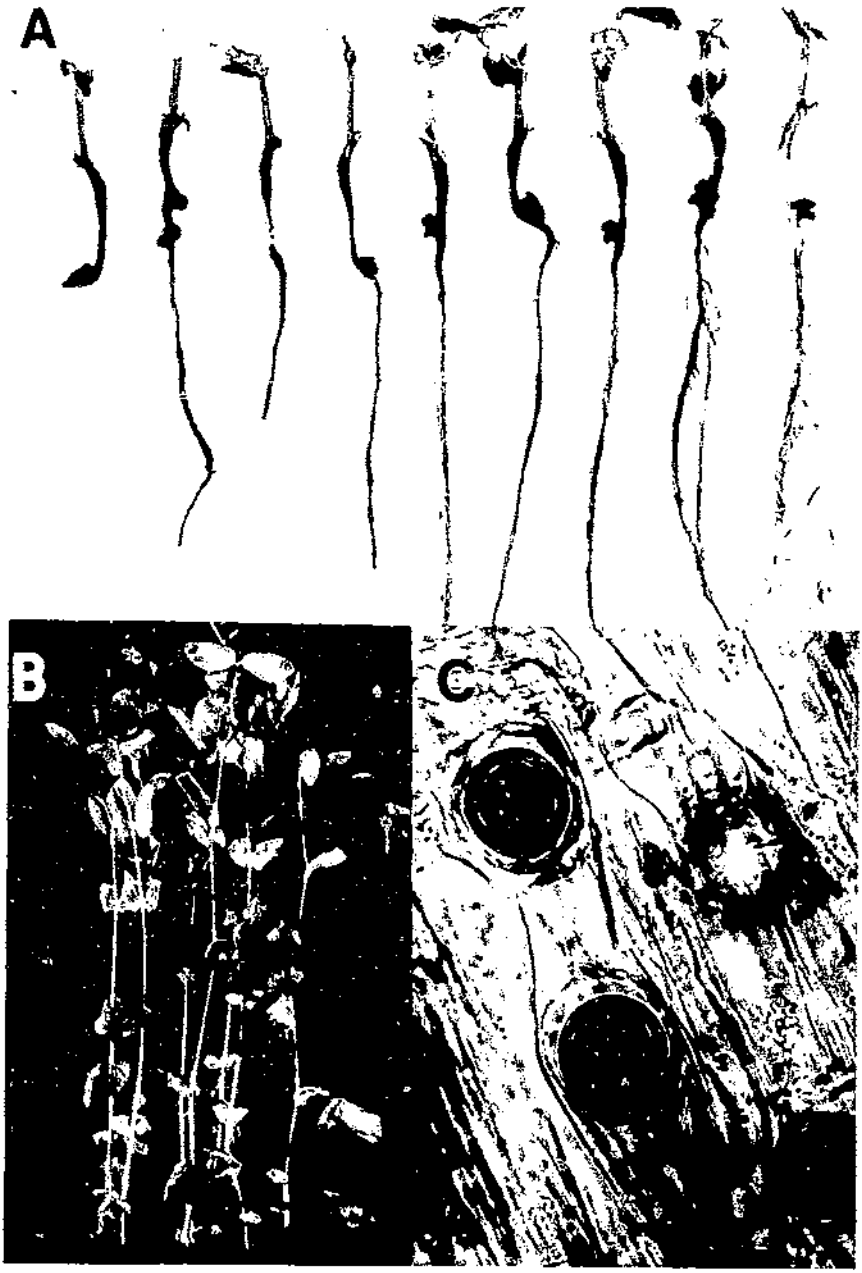
beet, sugarbeet, mangel, and chard, as well as several wild species of *Beta* (*B. maritima, B. patellaris*), were readily parasitized by *A. cochlioides* in the greenhouse and the field.

SYMPTOMS ON PEAS

Peas are susceptible to *A. euteiches* root rot throughout their life cycle. Infection occurs at any time during the growing season whenever environmental conditions are favorable. It may develop anywhere on the root or epicotyl. Symptoms of the disease are not characteristic but depend on the stage of development of the host when infection occurs.

Several early publications on *A. euteiches* fully describe the symptoms on peas (*126, 160, 161, 254, 289, 315*). The first symptoms of infection by *A. euteiches* may be discerned 3 to 4 days after penetration of roots and epicotyl. If the root and epicotyl of plants are examined in the early stages of infection, softened, water-soaked, and slightly discolored lesions may be seen in the cortical region (fig. 3, *A*).

From any point of entry the pathogen spreads rapidly, especially if the soil moisture is high, in all directions through the cortical tissue. As the decay progresses, the fine plant roots are destroyed.



PN-3579

FIGURE 3.—*A*, *Aphanomyces* root rot of 'Early Alaska' peas in the greenhouse. Note different stages of disease progression. Plant on right is healthy. *B*, Aboveground appearance of peas infected with *A. euteiches*. *C*, Oospores of *A. euteiches* in crushed cortex of infected pea root.

The water-soaked area, which resembles that caused by a bacterial infection, is firm at first and gradually becomes pale yellow to straw colored. The tissue becomes soft, especially in the epicotyl area, darkens with age, and eventually collapses and disintegrates (fig. 3, *A*). The dark color observed in the most advanced stages of the disease is due partly, if not entirely, to the invasion by secondary soil micro-organisms incapable of initiating infection of living, intact tissue by themselves. Such organisms may thrive on decomposing tissue and often give a reddishness to the vascular system of the plant. At this late stage *Aphanomyces* root rot cannot be easily distinguished from other forms of root rot.

Some of the symptoms may not develop on all plants. Depending on the environmental conditions and degree of tolerance of the cultivar, the root rot may only develop into a slight water-soaking on the epicotyls or on primary and secondary roots. In other instances, moderate water-soaking of primary roots or epicotyls may or may not be followed rapidly by extensive shrinking and darkening of the affected areas. In severe cases, the infected areas may be extensive and darkened and the tissue may collapse.

The soft rot may extend 2 to 5 cm. above ground, especially under humid conditions. Although *A. euteiches* does not penetrate the endodermis of mature roots (cf. Host-Parasite Interactions), it does cause the death of meristematic tissue at root tips. This inhibits subsequent root growth. In a few tolerant cultivars a protective secondary cortex is formed from a cambium developing in the pericycle. However, in most pea cultivars the endodermis does not appear to be an effective barrier against secondary invaders. Several species of *Fusarium* may enter the vascular tissue and quickly kill the plant.

The aboveground symptoms of the disease are not distinctive, except for the few centimeters of stem rot that may be observed above ground. Concurrently with the belowground development of the disease, the lower leaves may become yellow and brittle and this condition may progress upward (fig. 3, *B*). If infection occurs before the plant develops three to four nodes, under conditions favorable for the disease, sudden wilting may result. If infection is delayed until the roots have become well developed and adequate soil moisture has been maintained, the plants may appear normal up to harvest, producing poorly filled pods and low yields. In the field, infection takes place usually later than the three-to-four node stage, resulting in plants that are stunted and weakened. Occasionally during prolonged hot, dry weather most plants in an infested field may die before pods are filled. During wet weather,

however, a good crop may be obtained despite the severe rotting of the cortex.

In the greenhouse, and especially in the field, it may be difficult to distingiush *A. euteiches* root rot of peas from rots caused by other organisms. Two tests can be applied to obtain a decisive indication of this disease. If some of the infected plants are pulled, the vascular cylinder of the taproot pulls out readily from the decayed cortex as a long string, whereas roots of healthy peas almost always break at the seed attachment. This test does not work satisfactorily in early stages of the disease and in dry, compact soils. If superficial examination is not adequate for the detection of the disease, microscopic examination of the decayed cortex may reveal the presence of oospores of the pathogen (fig. 3, *C*). The oogonia of *A. euteiches* measure from 25 to 35 μ in diameter, and the sinuous inner surface and the smooth outer contour of the oogonia will help to distinguish *A. euteiches* from *Pythium* spp. The pulling of the vascular cylinder, presence of the sexual structures, and the softening of the cortex are the most conspicuous diagnostic characters that differentiate the disease from other root rots.

SYMPTOMS ON SUGARBEETS

Blackroot of table beets and sugarbeets caused by *A. cochlioides* is recognized in the field from poor stands. In addition, an abnormal downward curvature of the petioles (*231*), retarded plant growth, and general yellowing may sometimes reveal which plants are affected. Unaffected plants are erect, green, and vigorous. *A. cochlioides* is the most damaging root rot pathogen of these plants (*72*).

The blackroot disease complex, which starts in the very early seedling stage, occurs in two phases—an early acute phase of short duration and a later chronic phase that may persist throughout the life cycle of the plant. The acute phase is the more subtle and destructive since entire fields of 2- to 5-week-old plants may be destroyed. On young seedlings, symptoms were first observed and described by Peters (*245*), Edson (*105*), and Drechsler (*101*). A considerable amount of literature now exists describing symptoms on sugarbeets (*50, 56, 72, 143, 200, 316*).

The acute seedling phase of the disease may occur in two forms. First, there may be a preemergence damping-off, which results in death of seedlings after seed germination but before seedling emergence. This stage is extremely difficult to distinguish from

similar symptoms produced by other disease organisms (*Phoma betae*, *Pythium* spp., *Rhizoctonia solani*). Second, an early acute phase (postemergence damping-off) may be evident from the time of emergence until the first true leaves develop.

Infection of the hypocotyl occurs at the ground level. A water-soaked area extends up and down the hypocotyl or the upper part of the young taproot from the point of entry of *A. cochlioides* (fig. 4, *A*). Later the discoloration may extend up into the petioles

PN-3580

FIGURE 4.—*A*, Sugarbeet seedlings showing symptoms of blackroot (acute phase) caused by *Aphanomyces cochlioides*. Plant on right is healthy. *B*, Oogonia of *A. cochlioides* in collapsed sugarbeet seedling tissue (× 340). Note one with five antheridia. (Courtesy of C. L. Schneider.)

of the cotyledons. The invaded root or hypocotyl rapidly becomes brownish and then assumes the striking and characteristic jet black from which the term "blackroot" was apparently derived.

McKeen (*200*) stated that the term blackroot is somewhat of a misnomer and suggested that "black hypocotyl" would be more accurate. Shortly after the typical blackroot symptoms appear, the cortex of the hypocotyl dries and the stem and hypocotyl shrink to a dark, slender thread (fig. 4, *A*). Oospores of *A. cochlioides* can be easily seen by microscopic observations of the collapsed root and hypocotyl tissue (fig. 4, *B*).

Entire fields of sugarbeet seedlings may be destroyed by *A. cochlioides* within 3 to 4 days if warm, moist conditions prevail. If the weather is cool (about 10° to 12° C.) and moist, affected seedlings may survive for 2 weeks. The leaves are bluish at first and slowly turn yellow as the pathogen progresses up the stem. As the soil dries, and if the temperature remains low, new lateral roots may develop and the seedlings may recover. If a seedling survives and manages to produce three to five pairs of true leaves, the cortex of the hypocotyl may crack and the infected tissue may be sloughed off as a result of periderm formation below. At this stage the plant no longer suffers from the acute phase of the disease.

Buchholtz and Meredith (*50*) observed that the chronic phase of the disease first appears on older plants in late June to August. At this stage the pathogen stunts the plants and this condition results in undersized plants and considerable yellowing of the lower leaves. Wilting during the afternoons of sunny days and subsequent recovery (flagging) are common. First a greenish-yellow, later brown, and finally dark-brown, almost black, discoloration of the affected area characterizes the chronic phase. Infected tissues are slightly soft and split apart rather than tear when cut. The infected area appears slightly water-soaked and, when desiccated, shrivels to a "tassel" of vascular elements.

Kotila and Coons (*171*) attributed the growth reduction to the continuing attack by *A. cochlioides* on the lateral feeding rootlets. Plants with the chronic phase of the disease may appear dwarfed. Terminal parts of the taproot may rot if the soil moisture content is high. During the remainder of the growing season the plants either die or grow very slowly. Some of them may recover and grow satisfactorily during the remainder of the growth period. There is no distinct odor associated with rotted tissue during the chronic phase.

Schneider (*268*) induced the chronic phase of sugarbeet blackroot experimentally in the greenhouse with pure single-spore iso-

lates of *A. cochlioides*, originally isolated from diseased sugarbeet seedlings. Thirty-day-old plants were inoculated with 1×10^6 zoospores in 50 ml. of water per 3-inch pots. Chronic symptoms similar to those observed in the field developed within 6 weeks from inoculation time (fig. 5). At this later stage of plant growth, *A. cochlioides* induced severe wilting of the foliage, rotting and discoloration of secondary roots, decay of the terminal part of the taproot, and a generally reduced root size.

According to Coons et al. (*72*), the chronic effects of *A. cochlioides* are particularly serious on sugarbeets. The reduced size of the taproot lowers the quantity as well as the quality of the crop. The beets usually have a lower than normal sugar content and a higher impurity content. The latter makes sugar refining more difficult. The chronic phase appears to be particularly serious in the Northeastern United States (*72*). In contrast, the chronic phase of attack did not appear to be of great importance in southern Ontario (*200*). In a survey by McKeen (*200*), the chronic phase did not appear to affect more than 0.1 percent of the beets, even in areas where the acute seedling phase was most severe.

FN-35581

FIGURE 5.—Chronic phase of blackroot caused by *Aphanomyces cochlioides* on young sugarbeet plants. Plant on right is noninoculated control. Other four plants were inoculated with zoospores in the greenhouse. (Courtesy of C. L. Schneider.)

CAUSAL ORGANISMS

Isolation

Isolations from soil of *Aphanomyces* spp. associated with root diseases have been difficult and often frustrating. This difficulty may account for the rather late discovery that *Aphanomyces* spp. may cause root diseases. Prior to the use of antibiotics for the isolation of plant pathogens from soil, one of the biggest difficulties in isolating *Aphanomyces* spp. from putrescent plant tissue was the close association of its mycelium with bacteria, which are able to grow and proliferate on the surface of the *Aphanomyces* mycelium as it advances from the infected tissue outward on the agar surface (*101, 160*).

Acidification of the culture media and preliminary washing or surface treatment of the host tissue did not sufficiently help to separate the pathogen from the bacteria (*101*). The problem of bacterial contamination was especially acute when isolations were attempted from oospores of *Aphanomyces* in plant tissue, because of the longer time required for oospore germination and initiation of mycelial growth. Most of the early isolation techniques focused attention therefore on various practices to circumvent the bacterial contamination problem.

The original technique of isolation of *A. euteiches* was described by Jones and Drechsler (*160*). They used diseased plants in which *A. euteiches* was growing vigorously just before it formed oospores extensively in the tissue. Segments of pea tissue were selected in which the mycelium of *A. euteiches* could be seen under the microscope to be full of granular contents. The fragments were thoroughly washed in sterile water and placed on prune agar. Fragments of agar containing vigorously growing mycelial tips free of bacteria were then located and transferred to fresh agar. This procedure was repeated several times until cultures free of bacteria were obtained. Jones and Drechsler could find no satisfactory method to separate *A. euteiches* from *Pythium* spp. in their isolations.

The isolation technique of Jones and Drechsler (*160*) was subsequently modified as follows: After thorough washing, the fine lateral pea roots were placed in a petri plate with enough sterile water to cover them. Small segments, approximately 2 to 3 mm. long, free of contaminating fungi but possessing evacuated zoosporangia were cut out, passed through a few changes of sterile water, and placed on acidified commercial agar (*342*).

The ability of *A. euteiches* to produce motile zoospores was most ingeniously exploited by Sherwood (*348*) for the isolation of the

pathogen. The upper primary root of pea plants showing wilting of lower leaves was cut into 1-cm. segments. These were washed several times with sterile water and placed along the inside edge of a petri plate containing sterile tapwater. Autoclaved corn kernels were arranged opposite the infected pea root segments. Zoospores discharged from zoosporangia produced on the infected roots invaded the corn kernels within 4 to 12 hours. The kernels were then incubated for 8 to 12 hours each time in several changes of fresh sterile water to reduce bacterial proliferation. After 5 to 8 days of such changes they were removed with forceps, rinsed three times in sterile water, blotted dry between sterile filter paper, and plated on soil extract agar. *A. euteiches* hyphal tips were later transferred to soil extract agar or potato-dextrose agar. This technique may be useful for reducing or eliminating not only bacterial contaminants but also other saprophytic or parasitic fungi in the tissue.

A modification of the method used by Jones and Drechsler (*160*) to isolate *A. euteiches* was later devised by Drechsler (*101*) for the isolation of *A. cochlioides* from sugarbeet tissue. He first placed segments of infected material in 10 to 15 ml. of sterile water and changed the water several times at intervals to reduce bacterial contaminants. After adequate mycelial development from the segments, usually within 12 to 24 hours, the segments were removed, dried between blotting paper, and transferred immediately to cornmeal agar.

Drechsler's method of isolating *A. cochlioides* was further modified by Warren (*316*). Roots alone or infected hypocotyls without leaves were washed thoroughly in tapwater, rinsed twice in sterile water, placed in 20 ml. of sterile tapwater in test tubes covered with cotton plugs, incubated at room temperature for 24 to 28 hours, and examined for the presence of mycelium growing out of the infected tissue. The segments were then blotted dry between folded pieces of sterile paper towels and plated on cornmeal agar. Two other interesting modifications of the *A. cochlioides* isolation technique employing Van Tieghem cells were suggested by Warren (*316*) and by McKeen (*200*).

Since 1958–63, limited attempts have been made to employ antifungal or antibacterial antibiotics for the isolation of *Aphanomyces* spp. A technique to purify isolates of *A. euteiches* and *A. cochlioides* contaminated with bacteria was devised by Papavizas (unpub. data). The contaminated isolates were first cultured on cornmeal agar containing 50 mg. per liter of each chlortetracycline hydrochloride and streptomycin sulfate. Both species grew slowly, but after 1 week, transfers could be made from the edge of the

colonies to fresh medium containing the same antibiotics at 25 mg. per liter each. A second transfer from the antibiotic medium to cornmeal agar slants provided bacteria-free cultures. Neomycin was also used in a dilute V-8 juice agar to obtain single-spore isolates of phycomycetes, including *Aphanomyces,* from contaminated materials (*263*). Tolerance of *Aphanomyces* to streptomycin was also observed by Voros (*310*) and Sundheim and Wiggen (*300*).

Attempts to separate *Aphanomyces* from fungal contaminants presented a more difficult problem. Eckert and Tsao (*103*) tested 18 antibiotics on *Pythium, Phytophthora,* and *Aphanomyces.* Although both *A. euteiches* and *A. cochlioides* were strongly inhibited by pimaricin and high concentrations of nystatin, they grew slowly when only 25 mg. per liter of nystatin were used. Since this concentration is inhibitory to many fungal saprophytes, Eckert and Tsao suggested further experimentation with this antibiotic to isolate *Aphanomyces* spp.

Microscopic Detection

Several simple direct or indirect methods for observing fungal propagules in soil have been developed. Very little has been done, however, with respect to microscopic observations of *Aphanomyces* propagules in soil. Boosalis and Scharen (*44*) developed a direct microscopic observation technique for *A. euteiches* that may also be useful for *A. cochlioides.* The success of this technique is based on the characteristic morphology of *A. euteiches* oospores (*160*). Briefly, 100-gm. samples of soil were suspended in tapwater, the suspension was allowed to stand, and the supernatant liquid was poured onto a 60-mesh sieve. This procedure was repeated five to eight times until the supernatant liquid was relatively free of organic debris fragments. The plant debris retained on the sieve was gently rubbed for a few minutes, rinsed with tapwater, transferred to a 200-mesh sieve, rubbed and washed again to remove soil from the surface of the debris particles, and examined microscopically for oospores.

Later Scharen (*261, 346*) modified this method as follows: Large segments of pea roots screened from soil were transferred to a porcelain mortar and ground with a pestle. The macerated fragments were then put back on the 200-mesh screen and rinsed before the remaining procedure was performed. Dark materials difficult to examine with a microscope were covered with an aqueous solution of 5-percent sodium hypochlorite solution for 2 minutes and rinsed again on the 200-mesh sieve. Oospores observed by

Boosalis and Scharen and by Scharen in debris particles from peafields were morphologically similar to oospores produced on pure cultures or in roots of peas.

Mycelial Characteristics

The two saprolegnious plant parasites *A. euteiches* and *A. cochlioides* may be successfully cultured on nearly all media provided they contain the major ingredients needed for growth (cf. Physiology of Causal Organisms). The two species even grow out from substrates such as corn kernels submerged in water if there is a gradual leaking of nutrients from the substrate into the surrounding water (*341*). As the generic name implies (*Aphanomyces*= Greek aphanes+myces =obscure fungus), *Aphanomyces* appears in such water cultures as a very delicate, obscure, and almost imperceptible mycelial thallus radiating from the submerged substrate outward into the water. The mycelial thallus, as well as asexual and sexual stages of *Aphanomyces* spp., has been described in detail in a monographic study (*283*) and in the original publications describing *A. euteiches* and *A. cochlioides* (*101, 160*). Only the morphology of the two species will be given briefly here.

In macroscopic appearance, cultures of *A. euteiches* and *A. cochlioides* grow sparsely and in an arachnoid fashion on relatively poor media but have a high degree of uniformity and similarity on the same medium (fig. 6). On rich media, such as maltose-peptone agar, prune agar, and potato-dextrose agar, both organisms tend to produce a whitish aerial mycelium. On all solid media the vegetative hyphae ramify over and through the agar, forming a sparse arachnoid growth with little or no aerial development. After several weeks of vigorous growth, however, especially on rich media, the surface of the media becomes covered with thick, tough mycelial mats.

In liquid media the *Aphanomyces* spp. develop as a very delicate, almost imperceptible halo of filaments, which radiate from the inoculum source and extend outward into the surrounding liquid. Usually in 3 to 4 days at 25° C. an extensive, submerged mycelium is produced, which has the appearance of a translucent, white, nebulous mat. Although there are differences in macroscopic appearance among isolates of the same species, these differences are relatively small.

In microscopic appearance, the two species are very similar. *A. euteiches* and *A. cochlioides* possess mycelium 3 to 10 μ in diameter, delicate, hyaline, sparingly or moderately branched, and not fluctuating abruptly in thickness. Cultures of both organisms

PN-3582

FIGURE 6.—One-week-old cultures of *Aphanomyces euteiches* (above) and *A. cochlioides* (below). *Left*, potato-dextrose agar with 0.2-percent yeast extract; *right*, cornmeal agar without dextrose.

have a characteristic "*Aphanomyces*" odor. Except where zoosporangia and sexual structures are cut off, the thallus is coenocytic and grows straight without waviness.

Branching of the hyphae is almost at right angles (fig. 7). In addition to branches of indefinite length, other branches remain relatively short and thus form diverticulate spurs, which are especially frequent near the oogonia. Young, vigorous hyphae destined to become zoosporangia are normally packed with coarsely granular cytoplasm. Irregularly scattered vacuoles and smaller and refractive oil droplets may be found in the cytoplasm. In older hyphae a large, extensive, central vacuole may be surrounded by peripheral cytoplasmic contents. The hyphal walls are composed of cellulose that reacts positively with chloroiodide of zinc.

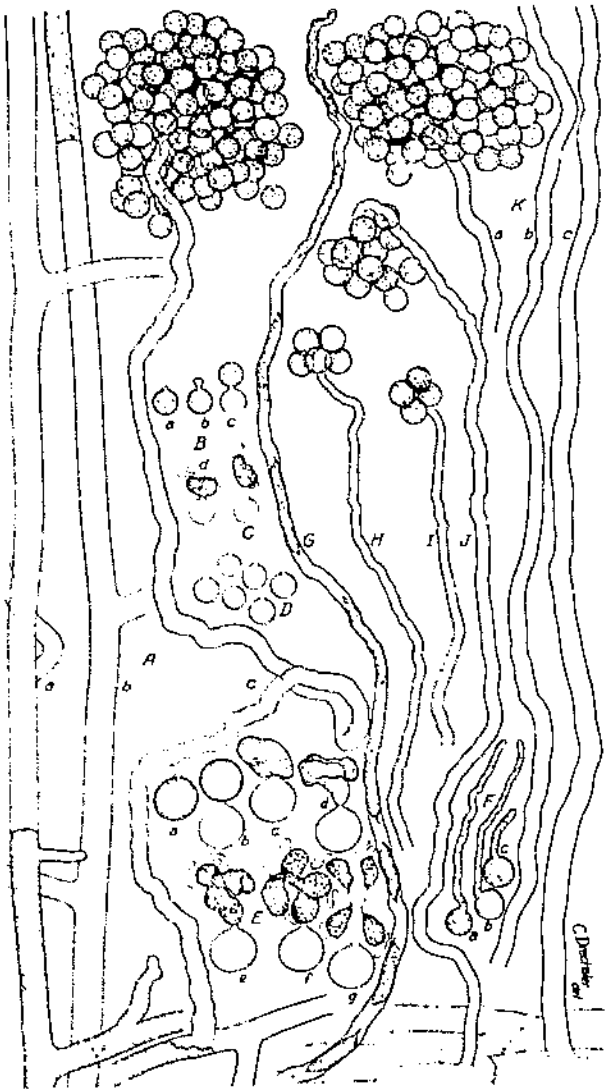


FIGURE 7.—Asexual reproduction of *Aphanomyces cochlioides* (camera lucida × 425): *A*, Parts of an evacuated sporangium developed from mycelium grown on cornmeal agar: *a*, Basal part of axial element; *b*, distal part; *c*, evacuation tube; *B*, successive stages (*a–d*) in evacuation of cyst membrane and development of motile zoospore *C*; *D*, evacuated cyst membranes; *E*, successive stages (*a–g*) in development of four zoospores from single abnormally large encysted structure; *F*, germination of zoospores (*a–c*); *G*, evacuation tube previous to discharge; *H–J*, small evacuation tubes after discharge arising from stem of a beet seedling; *K*, longer evacuation tube after discharge of more than 100 zoospores (*a–c*). (From Drechsler (*101*); courtesy of C. Drechsler.)

Within the diseased host, *A. euteiches* and *A. cochlioides* develop abundantly as hyaline, nonseptate, moderately branched mycelia. They are composed of hyphae varying considerably in diameter among themselves, but individually they are not subject to abrupt fluctuations in respect to dimensions. The axial filaments develop short diverticulate spurs, which exhibit only a slight tendency to penetrate host cells. The mycelium is largely intracellular and the hyphae are oriented longitudinally within the cells. Hyphal development between the plant cells appears relatively meager and rather accidental.

Asexual Stage

Several genera of the order Saprolegniales, including *Aphanomyces*, produce two types of zoospores. This phenomenon is called *diplanetism* (Gr. dis+planetes=twice+wanderer), and the organisms possessing this characteristic are *diplanetic*, or better, "dimorphic" (*283*). *Aphanomyces* has slender, filamentous zoosporangia with but a single row of zoospores, which behave on emergence as do those of the genus *Achlya*. No proliferation of sporangia is observed in the genus *Aphanomyces*, but plural evacuation hyphae may be formed in some species of the genus as a result of converting large segments of the thallus into zoosporangia.

To induce asexual reproduction in juvenile mycelium of *A. euteiches* and *A. cochlioides*, Jones and Drechsler (*160*) and Drechsler (*101*) used the common practice with water molds of obtaining vigorous mycelia on a suitable substratum, transferring the thallus to fresh water, and replacing the water successively a few times at about 15-minute intervals. The first visible evidence of asexual reproduction occurred 5 to 6 hours after mycelial washing was completed. For growth of *A. euteiches*, Jones and Drechsler (*160*) used pea decoction made by adding from eight to 10 pea seeds to 100 ml. of distilled water.

Under favorable conditions almost the entire thallus appears to become involved in asexual sporogenesis. The individual zoosporangia of *A. cochlioides* are very long, often extending 3 to 4 mm. They are sinuous, irregular in diameter, and involve large segments of the vegetative thallus (fig. 7). Although some tapering in diameter toward the apex of zoosporangia is usually perceptible, the pronounced attenuation characteristic of *A. euteiches* is very infrequent (*101*). Both axial and branching elements are delimited by septa, which may appear as regular cross walls (fig. 7, *A*, *a-b*) or as irregular partitions (fig. 7, *A*, *C*). The individual

zoosporangia in *A. euteiches* often consist of axial filaments from 1 to 2 mm. long and bearing from six to 10 well-developed branches, which taper distally to 4 μ.

The sequence of events occurring in vegetative growth and asexual reproduction of the two species, as described by various investigators (*101, 160, 200, 283*), may be divided into three distinct phases: (1) Vegetative growth of the thallus in a nutrient medium, (2) differentiation of primary zoospores within the zoosporangium, and (3) evacuation from and aggregation of encysted primary zoospores at the orifice of the zoosporangium. The second phase of the asexual sporogenesis begins when the young, vigorous thallus is transferred from the nutrient medium to pure water. First, the zoosporangial rudiments begin to fill with dense, granular protoplasm.

Studies (*154, 155*) of the ultrastructural changes in the vegetative hyphae of *A. euteiches* during differentiation of primary zoospores showed that, just prior to differentiation of primary asexual spores, the central vacuole of each filament is segmented by cytoplasmic strands, the electron-dense vesicular inclusions become enlarged and striated, and the nuclei move from a peripheral position to one near the longitudinal hyphal axis (fig. 8). Differentiation, which proceeds very rapidly, involves migration of cytoplasm around each nucleus, withdrawal of the plasmalemma from the hyphal wall, evagination of the central vacuole, and discharge of its contents into the space between plasmalemma and hyphal wall. The plasma membrane covering the primary spores is formed jointly by the tonoplast and the plasmalemma.

The irregularly shaped zoospore initials become separated from one another by their transverse bands of hyaline cytoplasm. As a result of the simultaneous segmentation of the contents of a zoosporangium and an apparent contraction of zoospores inside the sporangium, a single row of much elongated and sharply tapered zoospores is formed (*160*). These are connected by a delicate strand of tenuous protoplasmic material, which can easily be seen after evacuation has started. As they mature, zoospores become less elongated and protoplasmic threads disappear.

The third phase, zoospore evacuation, begins with the foremost zoospore being pushed against the tip of the filamentous sporangium, a typical "achlyoid" process. The latter suddenly ruptures at the tip and the first few zoospores are extruded with considerable speed. The zoospores upon discharge assume a spherical shape, secrete a cellulose wall, and accumulate at the orifice of the sporangium as an irregular mass (fig. 7). The number of zoospores liberated may vary from a few to 100, 200, and occasionally 300

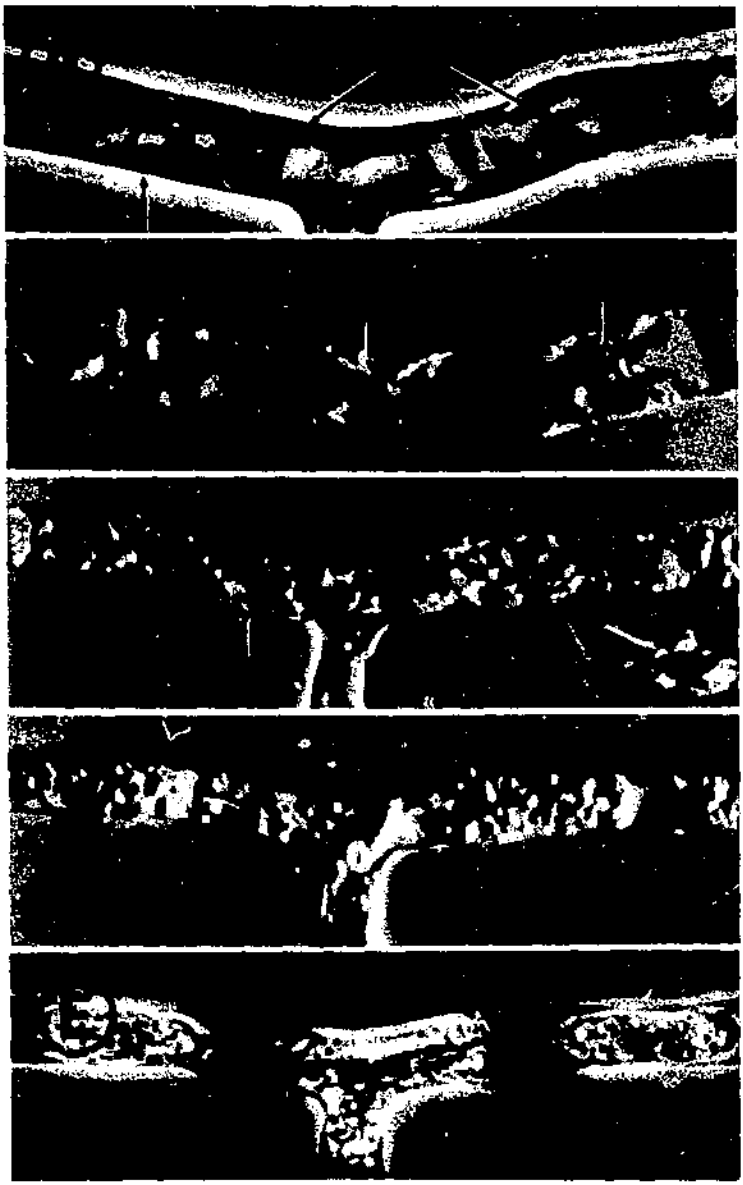


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FIGURE 8.—Hyphae of *Aphanomyces euteiches* in various stages of asexual reproduction: *A*, Cytoplasmic strands (*Cs*) extended across central vacuole (*V*); position of nucleus is indicated by arrow (phase contrast); *B* and *C*, aggregation of cytoplasm toward centered nuclei (arrows); *D*, hyphae with developing primary spores (Nomarski interference-contrast); *E*, hyphae with primary zoospore formation complete. (From Hoch and Mitchell (*155*); courtesy of J. E. Mitchell.)

or more per sporangium. The primary zoospore cysts may be from 8 to 11 μ in diameter in *A. euteiches* and 6 to 15 μ in *A. cochlioides*.

After a period of encystment usually lasting from 1 to 3 hours, secondary zoospores emerge from the primary zoospore cysts. First, a papilla about 2 μ in diameter appears on the cyst (fig. 7). The only morphological difference between *A. euteiches* and *A. cochlioides* in the asexual stage is in the diameter of the papilla through which the encysted spore is evacuated (*101*). In *A. euteiches* this dimension is one-fourth to one-third of the zoospore diameter, whereas in *A. cochlioides* it may only be equal to one-fifth. The papilla develops rapidly into a large hemispherical protuberance, which is eventually converted into a spherical vesicle by the streaming of the granular protoplasmic content from the cyst into the papilla. The vesicle is then disintegrated releasing a reniform secondary zoospore.

The secondary zoospores are about 13 μ long and 7 to 8 μ in diameter, but occasionally large double zoospores are observed. They possess two flagella, about 24 μ long, inserted in a slight depression of the zoospore. Cunningham and Hagedorn (*81*) showed details of zoospore flagellation of *A. euteiches*, a truly biflagellate phycomycete. The motile zoospores possess a whiplash and a tinsel-type flagellum (fig. 9). The whip (fig. 9, *a*) is at the tip of the flagellum. The flagellum on the right shows numerous tinsels along its entire length. Both flagella also show a "bubble" (fig. 9, *b* and *c*) in their crooks.

The secondary zoospores gradually increase their oscillating, swarming motion, and the lashing of their flagella becomes vigorous for a certain length of time (*200*). Zoospores of *A. cochlioides* may remain motile for 12 hours (*49*). After swimming about for variable lengths of time, usually about 3 to 4 hours, the secondary zoospores cease to be motile, lose their flagella, and round up. Under favorable conditions, germination may result in one to three germ tubes (fig. 7).

A continuous flow microtechnique for observing fine details of zoosporogenesis has been described (*156*).

When pea or sugarbeet root tissue, infected with the pathogen, is transferred to water, extramatrical mycelial filaments develop into zoosporangia in a few hours (*101, 283*). Primary zoospore cysts may easily be seen aggregated at the tip of the zoosporangia (fig. 10). Similar cyst aggregates appear at the tip of germinating *A. euteiches* oospores from debris particles. The zoospores produced may vary from very few to 300 or more per sporangium. McKeen (*200*) estimated that on an infected hypocotyl segment of sugarbeet 2.5 cm. long and about 2 mm. in diameter, as many as

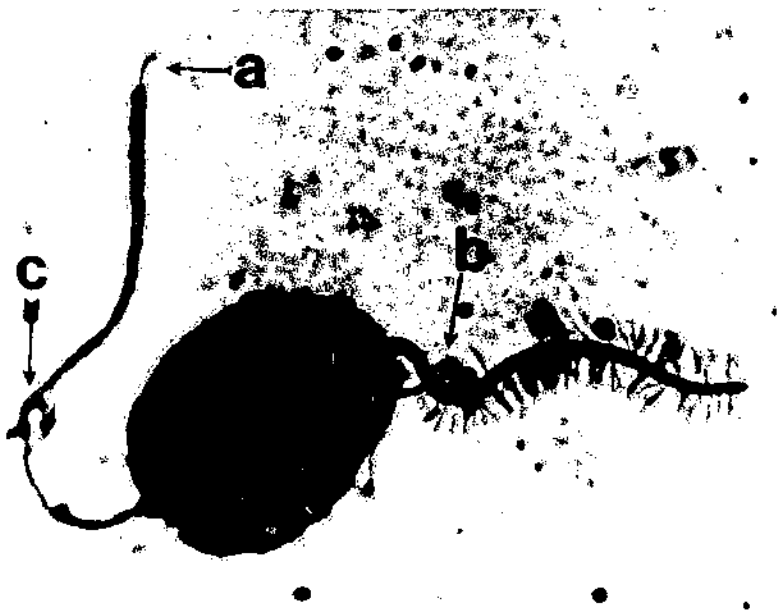

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FIGURE 9.—Biflagellate zoospore of *Aphanomyces euteiches*, showing whip (*a*) and bubble (*b*) in crook of flagella. (From Cunningham and Hagedorn (*81*); courtesy of J. L. Cunningham.)

500 evacuation tubes may develop, each discharging about 100 zoospores. Thus from the entire segment about 50,000 zoospores might be produced and released in a day into the surrounding environment. No one, however, has been able to observe zoospore formation from mycelial elements or directly from germinating oospores in nature.

Sexual Stage

Oospore formation is generally considered to occur when the thallus of *A. euteiches* and *A. cochlioides* is exposed to adverse conditions or environmental stresses (*160*). The purely vegetative condition represents a rather brief stage in the life cycle of the two species in infected pea and sugarbeet tissues and may come to an end as the infected tissues begin to collapse. The sexual reproductive organs, the female "oogonium" and the male "antheridium," appear on the vegetative mycelium. In agar or liquid media conducive to sexual reproduction, oogonia and antheridia may appear within a few days after transferring the pathogen to fresh media.

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FIGURE 10.—Filamentous zoosporangia and primary zoospore cysts of *Aphanomyces cochlioides* from diseased sugarbeet root tissue. (Courtesy of C. L. Schneider.)

In both pathogens the oogonia before fertilization are generally thin walled, subglobose to spherical bodies with densely granular, vacuolate contents, borne terminally on lateral branches of variable length arising from the vegetative thallus. Jones and Drechsler (*160*) described the oogonium of *A. euteiches* as follows: "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."

As in other species of *Aphanomyces*, the oogonial cavity is very large but not completely occupied by the single oospore. The

oogonia of *A. cochlioides* are also terminal on short, lateral branches, subspherical, 20 to 29 μ in diameter, averaging 24 μ, with the wall smooth at the outer surface and a sinuous inner contour. Both organisms possess a single "oosphere" or unfertilized "egg," which becomes an "oospore" upon fertilization. Oogonia are cut off from their concomitant hyphae by cross walls. The subtending parts are called "oogonial stalks."

In *A. euteiches*, antheridia are of diclinous origin, one to five in number, large, curved-clavate, borne on a stalk frequently involved with the oogonial stalk, branching once or several times, measuring 8 to 10 μ in diameter by 15 to 18 μ in length, and with visible fertilization tubes (figs. 11 and 12, *A*). If more than one antheridium is present, all or several may develop fertilization tubes. The antheridial cell itself may also be conspicuously arched and vermiform, somewhat lobulate, with fertilization tubes forming where the basal lobe touches the oogonial wall (fig. 12, *B*).

In *A. cochlioides*, from one to five antheridia, 6 to 10 μ in diameter and 9 to 18 μ in length, become wrapped about individual oogonia (fig. 13). The antheridia are club shaped with apical prolongations, separated from the stalk by a septum, with diclinous antheridial branches. Although the orientation of the oogonia and antheridia in *A. cochlioides*, as well as their morphology and development, shows great similarities to those of *A. euteiches*, the basal septum delimiting the oogonium in the former species never develops into a columella-like structure.

Because antheridial branches of variable length are present in *A. cochlioides*, the sexual apparatus in this species appears more complex than that in *A. euteiches* (figs. 12, *B*, and 13, *B*). The crowded, frequently "cochleate" condition of the antheridial apparatus is characteristic of *A. cochlioides*. In addition, the oogonial wall in *A. cochlioides*, though exhibiting fluctuations in thickness from point to point, is not sculptured on its inner surface as prominently as in *A. euteiches*. The contour of the oogonial wall of *A. euteiches* is often so sinuous that it gives the entire structure an internally scalloped appearance (fig. 11).

The only critical observations made on the fertilization process of *Aphanomyces* were those of Kasanowsky (*162*) in *A. laevis*. The actual fertilization process has never been observed in *A. euteiches* and *A. cochlioides*. The empty appearance of the antheridial cells and the conspicuous thickening of the oogonial wall signify that fertilization has taken place.

In *A. euteiches*, oospores are hyaline, subspherical or more rarely ellipsoidal, 18 to 25 μ in diameter (generally 20 to 23 μ), uniformly thick walled (1.2 to 1.8 μ), with a large central oil

globule surrounded by granular material (figs. 11, 12, *B*). A smaller refractive body is embedded in the granular material. Oospores in *A. cochlioides* are hyaline to yellow, 16 to 24 μ in diameter, or about 5 μ less than that of the oogonium, with granular contents, a large, central, reserve oil globule about 12 μ in diameter, and a smaller conspicuous refractive body. According to Drechsler (*100*), oospore walls are 1.5 to 2 μ thick, never 3 to 6 μ as given by Peters (*245*) for *A. laevis*. The oospores

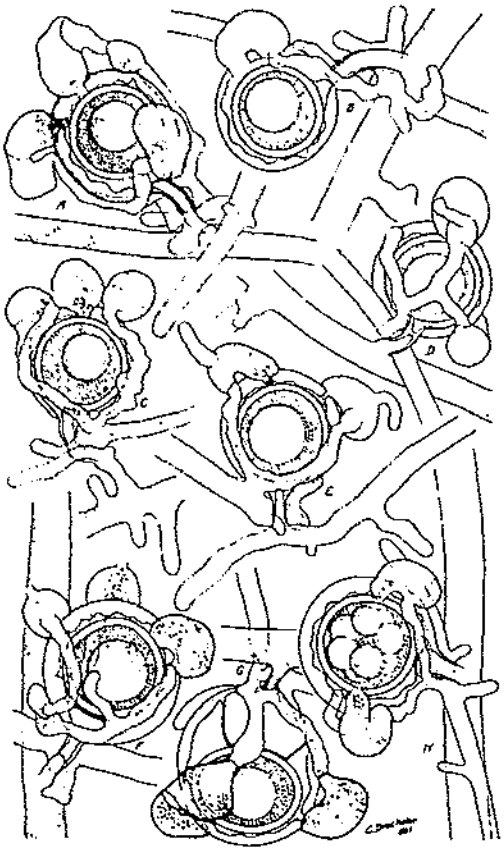

FIGURE 11.—Sexual apparatus of *Aphanomyces euteiches* from 20-day-old cornmeal agar cultures (camera lucida × 315): *A* and *D*, Terminal oogonium on short stalk and three antheridia intimately involved with oogonial stalk; *B* and *C*, short diverticulate branches borne on hyphae from which antheridial branches and oogonial stalk originate; *E* and *F*, hyphal diverticulum as dorsal appendage to antheridia; *G*, transverse septa often present at antheridial constrictions; *H*, sharply arched antheridia with fertilization tubes. (From Jones and Drechsler (*160*); courtesy of C. Drechsler.)

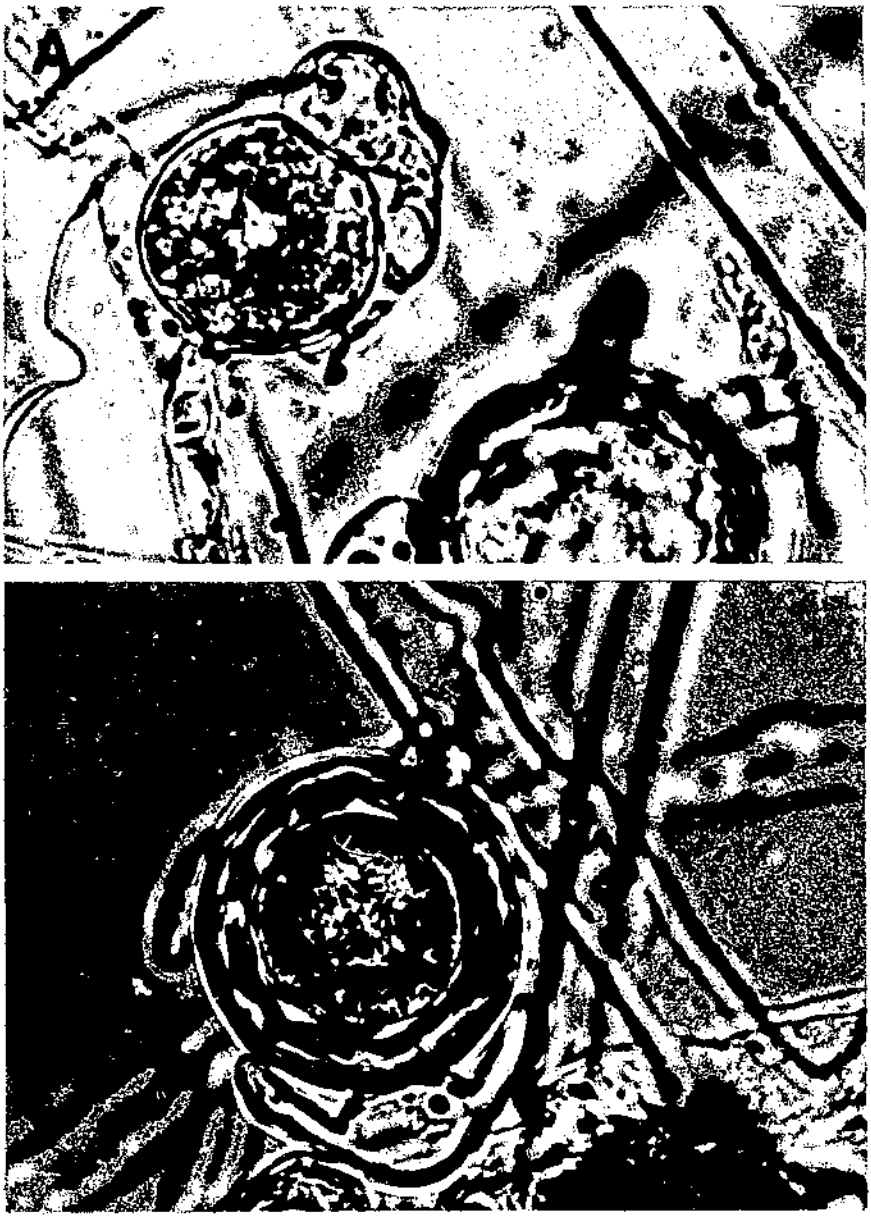

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FIGURE 12.—*A*, Oogonia and antheridia of *Aphanomyces euteiches* showing fertilization tubes; *B*, mature oospore of *A. euteiches* on cornmeal agar (× 1,350).

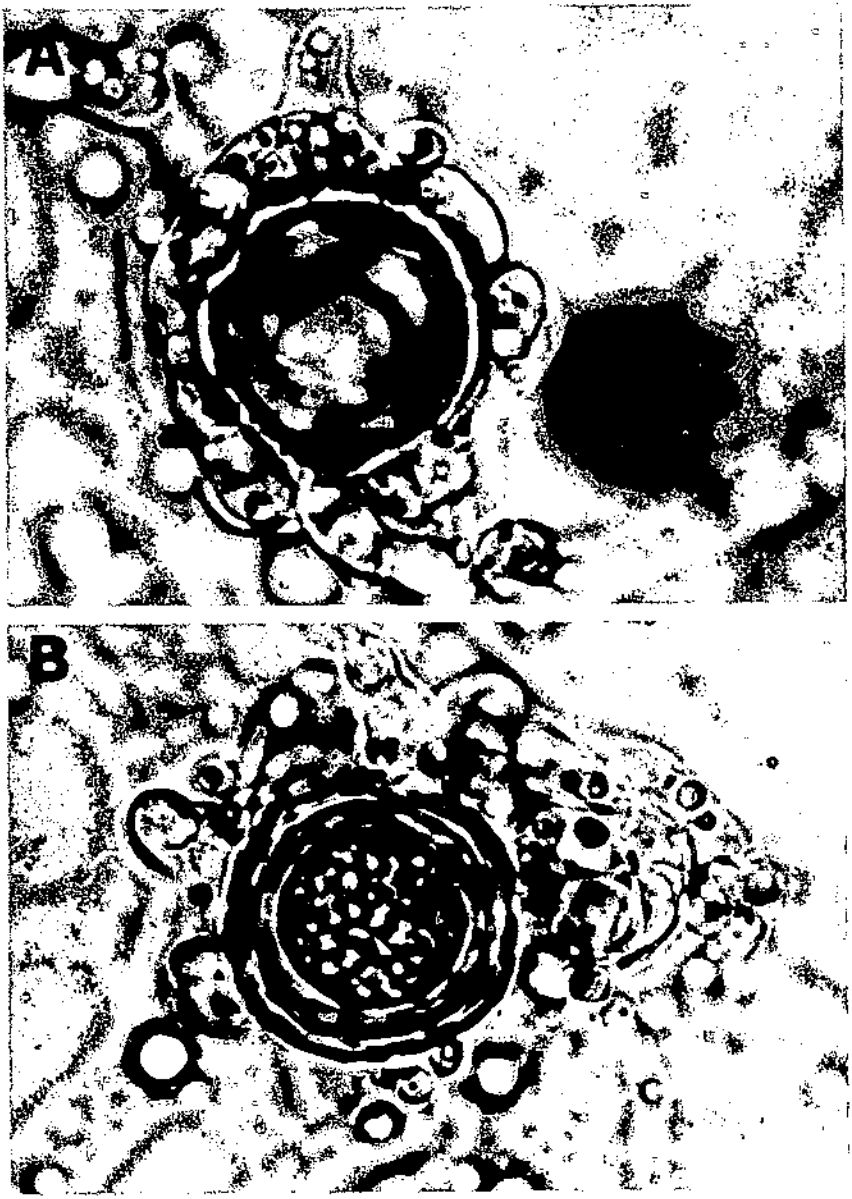

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FIGURE 13.—*A*, Oogonium and antheridia of *Aphanomyces cochlioides*; *B*, mature oospore of *A. cochlioides* on cornmeal agar. (× 1,350.)

of *A. cochlioides* fill the oogonia more completely than those of *A. euteiches*, but the diameter of the oogonia and the oospores is greater in *A. euteiches* than in *A. cochlioides*.

Cytology and Fine Structure

Far too little information is available on the cytology and nuclear condition of the *Aphanomyces* spp. Practically no data exist on the fine structure of *A. cochlioides*. The available information on the fine structure of the thallus of *A. euteiches* comes almost entirely from the University of Wisconsin (*154, 155, 284*).

Studies (*155*) of the ultrastructure of *A. euteiches* vegetative mycelium prior to zoospore differentiation showed that somatic hyphae contain a large central vacuole occupying the entire length of the hyphae except the 40 to 60 μm of the growing tip. Numerous vesicles with electron opaque inclusions can be observed in the somatic hyphae prior to differentiation (fig. 14, *A*).

Details of the nuclear structure and other fine structures of the somatic hyphae were furnished by Shatla et al. (*284*), who described the ultrastructure of tissue grown in microcultures on sterile glass slides. After an incubation period of 24 to 28 hours, the mycelial tissue was prepared and stained for electron microscopy. Shatla et al. observed numerous nuclei variable in shape, each with a prominent nucleolus and with an average diameter of 4.6 μ in the growing somatic hyphae. Nucleoli were very prominent under phase-contrast illumination. The mechanism of nuclear division in the somatic hyphae, however, was not observed.

Under the electron microscope the hyphal cell walls appeared as an amorphous electron transparent zone and the plasmalemma as a membrane lining the cell wall except where lomasomes were present under the plasmalemma (fig. 14, *B* and *C*). Endoplasmic reticulum, ribosomes, vacuoles, and dictyosomes (Golgi apparatus) were found in the endoplasm. Mitochondria were surrounded by a double membrane and the inner one formed swollen cristae (fig. 14, *C*). Unidentified microtubules and crystals were also found in the endoplasm and in vacuoles, respectively, for the first time by Shatla et al. (*284*). The unidentified structures observed by Shatla et al. (*284*) were later identified as plasmalemmasomes by Hoch and Mitchell (*155*).

Recently extruded zoospores of *A. euteiches* are bound by a single cell membrane (*155*). Many vesicles containing electron opaque striated inclusions may be seen within the dense zoospore protoplasm as well as bulging from the spore surface. In a few

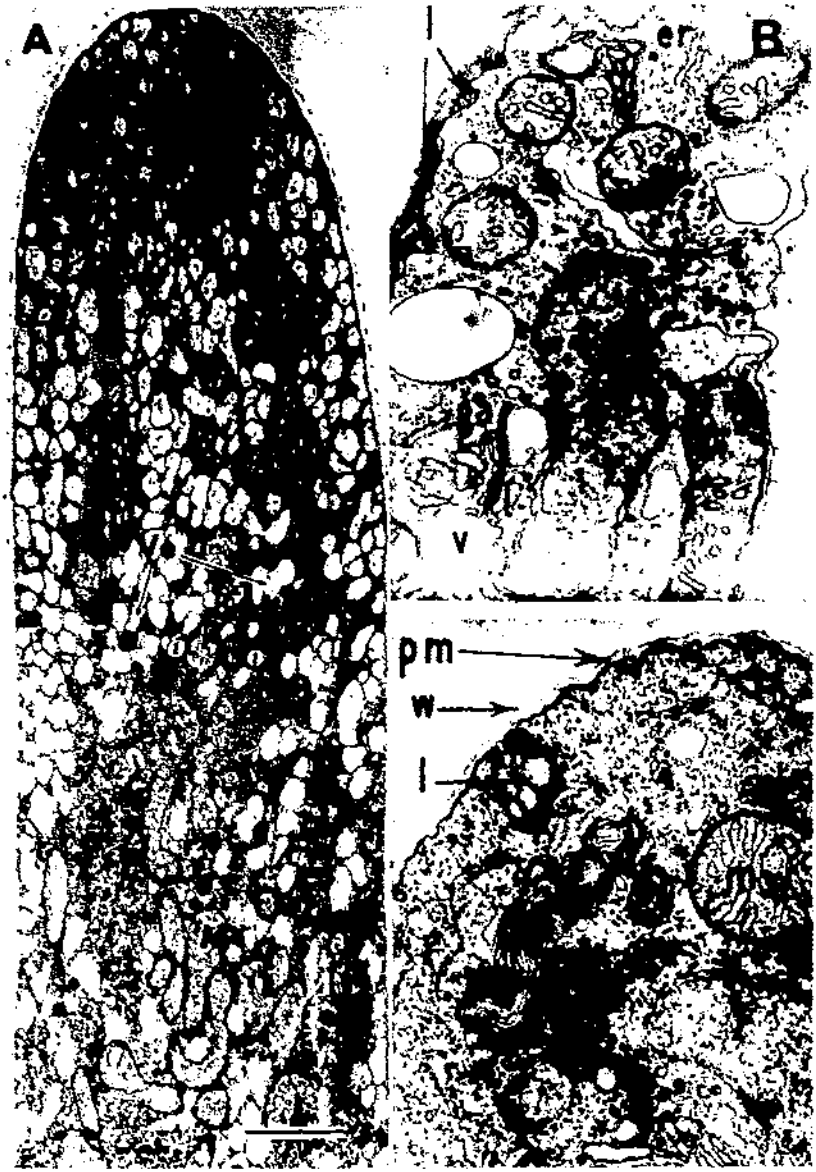

FIGURE 14.—Ultrastructure of vegetative hyphae of *Aphanomyces euteiches*: *A*, Near median longitudinal section of hyphal tip showing electron opaque inclusions (*Ve*) and mitochondria (*M*) (from Hoch and Mitchell (*155*)). *B* and *C*, Fine structure of somatic hyphae: *er*, endoplasmic reticulum; *ga*, Golgi apparatus; *l*, lomasomes; *m*, mitochondria; *pm*, plasmalemma; *r*, ribosomes; *v*, vacuole; *w*, cell wall (from Shatla et al. (*284*); courtesy of J. E. Mitchell).

hours the extruded primary zoospores develop cellulose cyst walls (fig. 15), which are characterized by a lighter inner zone and a darker outer zone. The older encysted zoospores contain a pear-shaped nucleus with a dense nucleolus, one or two kinestosomes near the tapered end of the nucleus, Golgi complexes around the nucleus, multivesicular bodies, and lipids. The endoplasm reticulum cisternae contain microtubules.

Some controversy exists in the literature with reference to the nature of the internal oospore elements. The large central body has been referred to sometimes as an oil globule (*65, 88, 283*). At other times, even in the same publications (*65*), it is regarded as being of protoplasmic nature and the granularlike structures as consisting of oily matter. Miller and King (*212*) showed that *A. euteiches* oospores give a positive reaction for

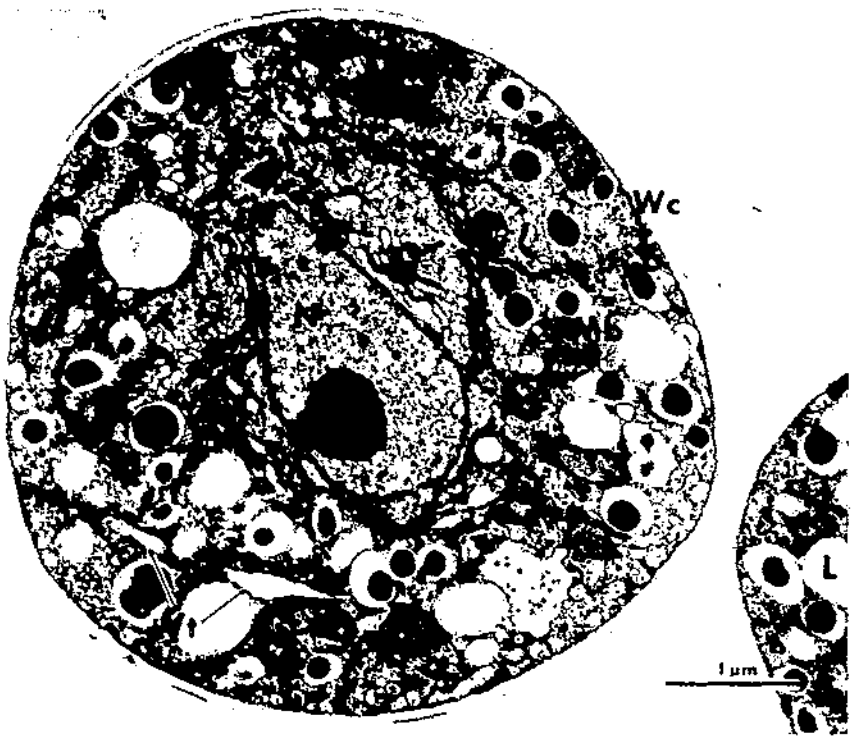

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FIGURE 15.—Encysted primary zoospores of *Aphanomyces euteiches* (× 18,200): *G*, Golgi complexes; *K*, kinestosomes; *L*, lipid; *Mb*, multivesicular bodies; *N*, pear-shaped nucleus; *Nu*, nucleolus; *Vt*, endoplasmic reticulum cisternae containing microtubules; *Wc*, cyst wall. (From Hoch and Mitchell (*155*); courtesy of J. E. Mitchell.)

deoxyribonucleic acid when stained with the Feulgen reagent. The uncertainties on the nature of the internal oospore matter have been perpetuated to the present time (*91, 157*).

Information on whether *A. euteiches* and *A. cochlioides* are homothallic or heterothallic is very meager. McKeen (*200*), who obtained normal sexual fruiting bodies of *A. cochlioides* in colonies resulting from single zoospores, concluded that the sugarbeet pathogen is homothallic. The nuclear condition of the zoospores, however, was not examined.

Taxonomy and Nomenclature

In a monograph on the genus *Aphanomyces* (*283*), 13 species were recognized as belonging to this genus. The specific epithets *A. euteiches* and *A. cochlioides* were maintained by Scott (*283*) as the valid names of the pea and sugarbeet parasite, respectively. Since Scott presented a complete account of the history of the genus *Aphanomyces* and the life cycle of its species, and since he critically evaluated and amply discussed the taxonomic criteria for separating species of *Aphanomyces*, there is no need here for further details on taxonomy and nomenclature.

PHYSIOLOGY OF CAUSAL ORGANISMS

Growth in Complex Media

Geach (*114*) recorded rapid mycelial growth of *A. euteiches* on prune, malt, potato, potato-sucrose, potato-dextrose, starch-asparagine, and cornmeal agars. Of these, the greatest density of mycelium was obtained on potato-dextrose, potato-sucrose, potato, and cornmeal media. Potato-dextrose agar (*218, 280, 348*) and maltose-peptone broth and agar (*187, 283, 338*) have been used extensively for routine propagation of the fungus. Cornmeal agar was observed by Jones and Drechsler (*160*) to support long survival of the organism, and this has been the medium used most frequently for maintaining cultures of *A. euteiches* (*114, 157, 238–241, 336, 341*). Growth of *A. euteiches* also occurs in 2-percent peptone in the absence of glucose or other carbohydrates (*218*).

According to Drechsler (*101*), *A. cochlioides* may be cultivated readily in nearly all kinds of commonly used artificial media. Cornmeal agar supported good growth, and viability was maintained in this medium even when cultures were transferred at yearly intervals (*101*). Schneider (*271*) used cornmeal agar for maintaining this species and 0.3-percent peptone broth for prop-

agating the mycelium for zoospore production. Maltose-peptone agar was found by Scott (*283*) to be suitable for the rapid development of the sexual stage.

Growth in Chemically Defined Media

Much of our understanding of the nutrition of *Aphanomyces* spp. stems from attempts to develop synthetic media. Whiffen (*319*) found that *Aphanomyces stellatus* deBary and four other species of the Saprolegniaceae made good growth in a glucose-glutamic acid-cystine medium. This finding served as a starting point for nutritional studies of *Aphanomyces* spp. by several workers.

Sherwood (*348*) obtained growth of *A. euteiches* on a glucose-glutamic acid-cysteine medium, but growth was not as abundant as in this medium when supplemented with yeast extract. Haglund et al. (*137*) developed a medium composed of glucose, asparagine, methionine, and mineral elements, which permitted good growth (table 3). Of 37 amino acids tested in place of methionine in this medium, only cystine, homocystine, and cysteine supported growth. No growth was obtained when ammonium nitrogen or nitrate nitrogen was used in place of amino nitrogen (*137*).

During a nutritional study of *A. euteiches,* Papavizas and Davey (*238*) developed a synthetic medium (table 3) that supported abundant growth. This medium, which contained DL-glutamic acid as the chief nitrogen source along with reduced sulfur as thioglycolic acid, did not permit growth as rapid as that in a complex medium containing yeast extract. Substitution of a mixture of amino acids, similar in composition to the amino acids present in commercial yeast extract for glutamic acid, supported a growth rate approaching that in the complex, undefined medium.

Later a medium similar in some respects to that of Haglund et al. (*137*) was developed by Yang and Schoulties (*329, 330*) (table 3). They (*330*) believed this to be superior to several media tested for vegetative growth. The mycelial growth rate of *A. euteiches* in this medium was similar to that in a peptone medium, and mycelial weights at the end of the incubation period exceeded those of the fungus grown in the peptone medium.

A. cochlioides was reported by Winner (*324*) to grow well in the glucose-glutamic acid-thioglycolic acid medium used by Papavizas and Davey (*238*) for *A. euteiches.* Winner found that mycelial dry weights of *A. cochlioides* were increased by substitution of D-, L-, or DL-methionine (30–120 mg. per liter) for thioglycolic acid as a sulfur source in this medium. Fowles (*340*)

TABLE 3.—*Composition of some synthetic media used by several investigators for culturing* Aphanomyces euteiches

Haglund et al. (*137*)			Papavizas and Davey (*238*)[1]			Yang and Schoulties (*330*)		
Ingredient[2]	Amount per liter		Ingredient[2]	Amount per liter		Ingredient[2]	Amount per liter	
Glucose	gm	5	Glucose	gm	5.4	Glucose	gm	5.5
L-Asparagine	gm	.75	DL-Glutamic acid	gm	1.51	DL-Asparagine	gm	4
L-Methionine	mg	95	Thioglycolic acid	ml	.28	Glutathione	gm	.1
Potassium phosphate, monobasic (KH_2PO_4)	gm	2	Potassium phosphate, monobasic (KH_2PO_4)	mg	68	Potassium phosphate, monobasic (KH_2PO_4)	gm	.7
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	mg	50	Sodium phosphate, dibasic (Na_2HPO_4)	mg	23	Potassium phosphate, dibasic (K_2HPO_4)	gm	.4
Manganese chloride ($MnCl_2 \cdot 4H_2O$)	mg	5	Calcium (as $CaCl_2$)	mg	40.1	Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	gm	.4
Ferric chloride ($FeCl_3$)	mg	5	Magnesium (as $MgCl_2$)	mg	48.6	Calcium chloride ($CaCl_2 \cdot 2H_2O$)	mg	20
Zinc chloride ($ZnCl_2$)	mg	5	Micronutrients[3]					

[1] A later modification of this medium contained increased levels of glucose (16.2 gm. per liter) and glutamic acid (3 gm. per liter) and had DL-methionine (150 mg. per liter) substituted for thioglycolic acid (*85, 237*).

[2] pH for media used by these 3 groups of investigators=5.5, 6.2, and 6.4, respectively.

[3] Amounts in milligrams per liter: Iron (as $FeCl_3 \cdot 6H_2O$), 11; zinc (as $ZnCl_2$), 0.7; copper (as $CuCl_2$) and boron (as H_3BO_3), 0.01; manganese (as $MnCl_2 \cdot 4H_2O$), 0.1; molybdenum (as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$), 0.02.

used a medium consisting of D-glucose, DL-glutamic acid, L-cysteine, and minerals for culturing *A. cochlioides*.

Nutritional Requirements of *Aphanomyces* spp.

Although the minimal requirements for vegetative growth of *Aphanomyces* spp. have not been completely defined, considerable progress has been made in establishing some of the exogenous nutrient requirements of these fungi in vitro. These, in turn, may lead to an understanding of some of the physiological responses of the organisms and their behavior during pathogenesis.

Nitrogen Sources Utilized

Amino nitrogen is the most readily utilized source of nitrogen by *A. euteiches* and *A. cochlioides*. Peptone supports abundant vegetative growth of both species. Asparagine or glutamic acid satisfies the nitrogen requirement of *A. euteiches* (*137, 238*) and *A. cochlioides* (*324*) when other nutritional requirements have been met. Glutamine or aspartic acid can also serve as the sole nitrogen source for *A. euteiches* (*197*) and *A. cochlioides* (*340*). However, growth is generally less rapid with these single amino acids than with an amino acid mixture or with peptone (*238*).

Inorganic nitrogen in the form of ammonium salts is apparently utilized by *A. euteiches*, although various isolates of this species may differ in this ability (*335*). Haglund et al. (*137*) reported that ammonium nitrogen as principal nitrogen source did not support the growth of one isolate. On the other hand, Papavizas and Davey (*238*) found that ammonium chloride supported growth of two isolates when other nutritional requirements were satisfied. However, growth was much curtailed unless the pH during growth was maintained by periodic adjustment within an optimal range (pH 5.5–7). Their data indicated that appreciable growth supported by ammonium nitrogen was related to the effective buffer capacity of the medium (fig. 16). Growth of *A. euteiches* in media containing ammonium chloride as the sole nitrogen source and with careful pH control, however, was less than in a medium containing amino nitrogen (*238*). Ammonium chloride also supported fair growth of *A. cochlioides* in the study by Fowles (*340*).

Nitrate nitrogen, as a general rule, is not utilized by either *A. euteiches* (*137, 238*) or *A. cochlioides* (*324*). Carley (*335*) noted one exception. Out of 13 of his isolates of *A. euteiches*, 1 was apparently able to derive its nitrogen from calcium nitrate. This general inability to utilize nitrate nitrogen was

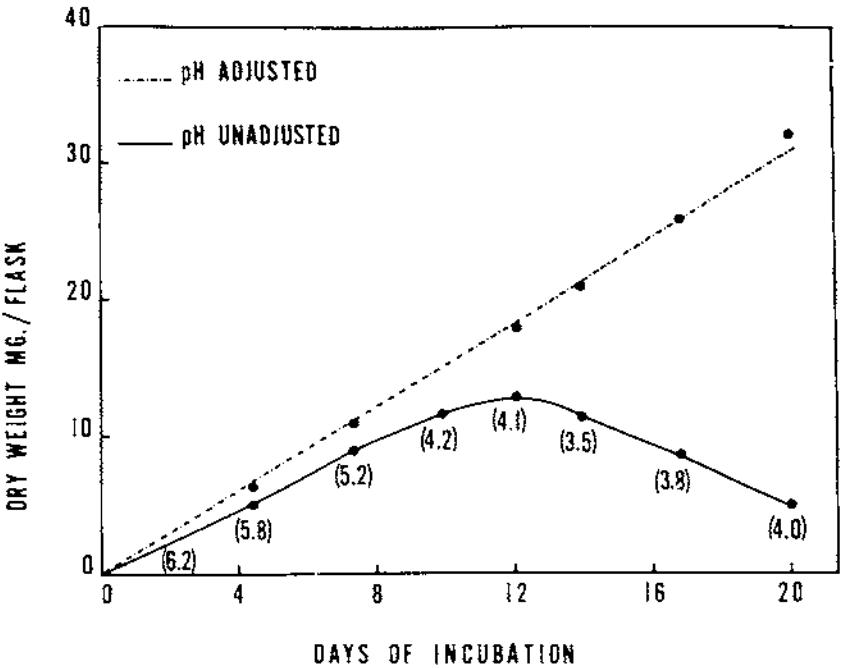

FIGURE 16.—Growth curves of *Aphanomyces euteiches* in synthetic medium with ammonium chloride as sole nitrogen source. Numbers in parentheses are pH values of unadjusted medium after indicated period of growth. (From Papavizas and Davey (*238*).)

noted by Cantino (*58*) to be true of most members of the Saprolegniales.

Carbon Sources Utilized

The carbon and energy requirement for growth of *A. euteiches*, along with its nitrogen and sulfur requirements, can be satisfied by peptone (*218*), peptides (*328*), or casein hydrolysate (*218*). Individual amino acids, such as glutamic acid, when supplied along with a suitable sulfur source can support limited growth of the fungus (*238*). Thus this organism possesses the enzymatic system(s) to derive energy and carbon for synthesis and growth from the oxidation of at least certain amino acids. Nevertheless growth of *A. euteiches* is greatly enhanced by carbohydrates and they are apparently the preferred sources of energy and carbon.

In a study of 43 carbon sources tested for their ability to support growth of *A. euteiches* in a medium adequately supplied with appropriate nitrogen and sulfur sources, about eight, chiefly mono-

saccharides and disaccharides, were unequivocally utilized (*237*) (table 4). Glucose and maltose were the best carbon sources, followed by glycerol, galactose, fructose, ribose, and cellobiose. Compounds that failed to support growth included a variety of sugar acids, sugar alcohols, polysaccharides, sugar esters, and certain monosaccharides and disaccharides. Interestingly *A. euteiches* was unable to use certain monosaccharides, such as mannose alone, but did so when this sugar was supplied along with glucose or galactose in the medium. Apparently *A. euteiches* is able to utilize adaptively certain sugars if a suitable carbon source is immediately available for growth.

The carbon nutrition of *A. cochlioides,* like several other aspects of its physiology, needs investigation. There are no reports of the carbon compounds that can serve as energy sources for this species in defined media.

Sulfur Sources Utilized

An important facet of the nutritional pattern of all *Aphanomyces* spp. studied closely thus far is their inability to utilize sulfates as sole sources of sulfur. That this may be a general characteristic common to all Saprolegniales was also suggested by Cantino (*53*).

Sherwood (*348*) successfully grew *A. euteiches* in a synthetic medium containing cysteine along with glucose and glutamic acid as organic constituents. Since growth did not occur in a medium containing ammonium nitrate and magnesium sulfate as sole sources of nitrogen and sulfur, he suggested that organic sources of these elements might be required for growth. Haglund et al. (*137*) extended the list of sulfur compounds supporting growth to include the amino acids methionine, homocystine, and homocysteine. Papavizas and Davey (*238*) observed no growth of three isolates of *A. euteiches* in the absence of reduced sulfur. Either thioglycolic acid or methionine satisfied the sulfur requirement, but methionine appeared superior in stimulating growth.

Haglund (*341*) and Haglund and King (*139, 142*) reported that sodium thiosulfate ($Na_2S_2O_3$) and thiourea, in addition to the compounds previously cited, satisfied the requirement for 10 isolates, but growth failed with sodium bisulfite ($NaHSO_3$), sodium metabisulfite ($Na_2S_2O_5$), sodium dithionate ($Na_2S_2O_6$), or sodium sulfate (Na_2SO_4) supplied as sole sulfur sources. This clearly indicated that either organic or inorganic sulfur compounds having sulfur in the reduced state were suitable sulfur sources.

TABLE 4.—*Effect of various carbon sources on growth of* Aphanomyces euteiches *in basal medium 1 containing methionine as sulfur source* [1]

Carbon source [2]	Mycelial dry weight of indicated isolate per flask			
	A4	A7	A12	Average
	Mg.	*Mg.*	*Mg.*	*Mg.*
Monosaccharides:				
D-Glucose	147	121	169	146
D-Galactose	51	47	56	51
D-Fructose	37	25	50	37
D-Ribose	15	16	23	18
D-Mannose	1	2	4	2
L-Sorbose	0	0	0	0
D-Arabinose	2	<1	0	1
L-Arabinose	1	<1	2	1
D-Xylose	0	0	0	0
None	1	0	0	<1
Disaccharides and trisaccharides:				
D-Glucose (control)	154	127	159	147
Maltose (hydrate)	125	104	155	128
Cellobiose	18	24	25	22
Melibiose (hydrate)	4	5	1	3
Sucrose	1	3	2	2
Lactose	0	0	0	0
Raffinose (hydrate)	2	1	2	2
None	1	1	0	<1
Polysaccharides:				
D-Glucose (control)	151	112	160	141
Dextrin [3]	37	28	25	30
Soluble starch [3]	27	23	23	24
Glycogen	5	4	6	5
Carboxymethylcellulose [3]	2	1	3	2
Xylan [3]	2	1	3	2
Inulin [3]	1	1	2	1
None	1	<1	0	<1
Carbohydrate derivatives:				
D-Glucose (control)	145	101	163	136
Glycerol	45	42	68	52
Dulcitol	1	2	0	1
Mannitol	2	3	2	2
Sorbitol	2	1	2	2
Inositol	0	0	0	0
L-Rhamnose (hydrate)	3	2	2	2
Gluconic acid	0	0	0	0
Glucuronic acid	0	0	0	0
Saccharic acid [2]	0	0	0	0
Salicin	0	0	0	0

See footnotes at end of table.

TABLE 4.—*Effect of various carbon sources on growth of* Aphanomyces euteiches *in basal medium 1 containing methionine as sulfur source* [1]—Continued

Carbon source [2]	Mycelial dry weight of indicated isolate per flask			
	A4	A7	A12	Average
	Mg.	*Mg.*	*Mg.*	*Mg.*
Carbohydrate derivatives—Con.				
α-Methyl-D-glucoside	1	0	2	1
α-Methyl-D-mannoside	0	0	0	0
Methyl-α-D-glucopyranoside	0	0	0	0
None	1	0	1	1

[1] From Papavizas and Ayers (*237*).

[2] 5 gm. of carbon was added per liter of medium.

[3] Compound autoclaved separately and added aseptically to Millipore-filtered medium; other compounds sterilized with Millipore filters.

The sulfur nutrition of 12 isolates of *A. euteiches* was clarified further by Davey and Papavizas (*85*). They showed that compounds with sulfur in varying states of oxidation, or valence, from 0 to −2 were able to support growth, whereas sulfur in higher oxidation states of +4 or +6 would not. Thus thioglycolic acid, methionine, and other sulfur amino acids, all of which have sulfur with an oxidation number of −2, supported growth, whereas sodium sulfite (Na_2SO_3, oxidation number of +4) and Na_2SO_4 (oxidation number +6), did not.

Sodium thiosulfate ($Na_2S_2O_3$), which possesses an outer reduced sulfur and an inner oxidized sulfur with an oxidation number of +6, was utilized by two isolates of *A. euteiches*. Their growth resulted in the discriminate utilization of the outer reduced sulfur and in the accumulation of the inner oxidized sulfur as sulfate. Additionally it was established that elemental sulfur (oxidation number 0) was able to satisfy the sulfur requirement. Although the insolubility of elemental sulfur precluded its use where uniform growth was desired, *A. euteiches* grew around clumps of the element in liquid and on agar media.

The nature of the sulfur requirement of *A. cochlioides* is apparently very similar to that of *A. euteiches*. Both Winner (*328*) and Fowles (*340*) found that *A. cochlioides* required a reduced sulfur source for growth. Sodium sulfate (Na_2SO_4) was not utilized. L-cysteine, L-cystine, or L-methionine was superior to thioglycolic acid in supporting growth (*340*). The D as well as the L isomer of methionine was a satisfactory sulfur source for

A. cochlioides, although slightly higher yields of mycelial weight were achieved with the L isomer or the DL racemic mixture (*323*).

Mineral Requirements

Very little is known of the mineral elements required for growth by *Aphanomyces* spp. There has been no systematic attempt to determine individual inorganic nutrient requirements of the species, although such a study was made of several isolates encompassing several genera of the family Saprolegniaceae (*257*). On the basis of this and other fungal nutritional studies it may be presumed that sources of calcium, magnesium, manganese, and iron ions are probably required for normal growth.

A requirement for calcium ion for vegetative growth of *A. euteiches* was reported by Yang and Schoulties (*329*). The requirement could not be satisfied by zinc. Although a calcium source was not present in the synthetic medium of Haglund et al. (*137*) (table 3), inclusion of calcium chloride in this medium promoted earlier growth of *A. euteiches* (*29*). It seemed likely that sufficient calcium is present in other medium ingredients or inoculum to permit minimum growth where it is not deliberately supplied.

Papavizas and Davey (*238*) found that salts of magnesium, iron, and zinc supplied together supported growth of *A. euteiches,* whereas in their absence growth did not occur. Thus one, two, or all of these elements are required; the essentiality of the separate ions was not established. Careful inorganic nutritional studies with highly purified ingredients and metal chelators may be necessary to establish the essentiality of these or other elements for growth of *A. euteiches* and *A. cochlioides.*

Vitamins

Both *A. euteiches* and *A. cochlioides* apparently are autotrophic in respect to vitamins or accessory growth factors. Abundant growth of either species occurs in synthetic media without vitamins (*238, 325, 340*). Moreover, in the only study in which several known vitamins of the B group and vitamin C were deliberately supplied to a synthetic medium, growth of *A. euteiches* was not stimulated (*238*).

Effect of pH on Growth

Smith and Walker (*289*) studied the effect of initial pH of the culture medium on radial growth of *A. euteiches* using phos-

phate-buffered potato-dextrose agar. The pH limits of growth were about pH 3.4 and slightly above pH 8. The optimum range was between pH 4.5 and 6.5. With a liquid synthetic medium maintained at various pH values by periodic adjustment with acid or alkali, Papavizas and Davey (*238*) recorded the optimum pH range of one isolate of *A. euteiches* to be between 5.4 and 6.5. Growth at pH values above and below these levels fell off sharply. The optimum pH level for growth of *A. cochlioides* was recorded as between 6 and 7 (*340*).

Effect of Temperature on Growth

The temperature limits for vegetative growth of *A. euteiches* on potato-dextrose agar are about 12° and 32° C. The optimum was 28° in two studies (*187, 289*). This contrasted with the optimum temperature for zoospore production, which was about 4° below that for vegetative growth (*187*). In a carefully controlled temperature study by McKeen (*200*), the limiting temperatures for radial growth of *A. cochlioides* on potato-dextrose agar were 8° and 35°. Growth was good from 16° to 32°, with an optimum centered at about 29°. Fowles (*340*) noted a closely similar optimum range for this species. Like *A. euteiches, A. cochlioides* produced zoospores maximally at 24°, slightly lower than the optimum temperature for vegetative growth (*200*).

Effect of Oxygen Tension

Sherwood and Hagedorn (*286*) studied the effect of O_2 (oxygen) tension on growth of *A. euteiches*. Radial growth on potato-dextrose agar occurred more rapidly under an artificial atmosphere containing 5 percent of O_2 than under an atmosphere of 20 percent of O_2. Mycelial dry weights in a liquid medium were also greater at the lower O_2 tension. However, there was little growth at 0.3 and 0.01 percent of O_2. Their experiments indicated that the organism was an obligate aerobe, and yet it grew better at an oxygen tension below that of the normal atmosphere.

Other Factors Affecting Growth

Papavizas and Ayers (*237*) observed that glucose autoclaved within a medium supported more abundant growth of three isolates of *A. euteiches* than glucose sterilized by filtration through Seitz, membrane, or fritted-glass filters. Mycelial yields in Seitz-filtered media were consistently and substantially lower than in media sterilized by other means. Certain batches of Seitz filters appeared to contain a water-soluble material that was weakly

toxic to the fungus. However, there was no clear explanation for the greater growth of the fungus in autoclaved media in comparison with that in media sterilized by filtration through fritted-glass and membrane filters. It was suggested that glucose autoclaved within the medium might undergo some unknown chemical change that enhanced growth of the fungus.

Certain amino compounds interfere with the nitrogen metabolism of *A. euteiches* and affect its growth. β-Methylaspartic acid, an uncommon amino acid effective in suppressing *Aphanomyces* root rot of peas in several studies (*234, 236, 242*), was found by Lumsden et al. (*197*) to prevent germination and growth of the fungus in vitro. Concentrations as low as 0.025 mM. of β-methylaspartic acid markedly reduced growth of *A. euteiches* in a synthetic medium with ammonium chloride supplied as the only other nitrogen source at a 50-mM. concentration. β-Methylaspartic acid at 1 mM. completely suppressed growth.

A reversal of the growth inhibition occurred when aspartic acid, glutamic acid, asparagine, or glutamine was substituted for ammonium chloride in the medium. The kinetics of this reversal was suggestive of competitive inhibition by β-methylaspartic acid of some metabolic step involving one or more of these amino acids within the fungal cells. β-Methylaspartic acid at concentrations as low as 1 mM. also prevented zoospore germination. The inhibitory effect of β-methylaspartic acid on mycelial growth from zoospores was reversed when appropriate concentrations of 5 to 10 mM. of glutamic acid were added to the medium.

Intermediary Metabolism and Respiration

Little is known of the intermediary metabolism of these fungi. With carbohydrates as chief carbon sources, some acidic products are released into the medium during growth of *A. euteiches*, as indicated by a slow drop in pH (*238*); however, there is apparently no great outpouring of organic acid intermediate (or end products) that is characteristic of many fungi. Likewise during growth in media containing peptones or amino acids without carbohydrates, a slight alkaline reaction results, presumably through the release of some ammonia. There is little evidence of end-product accumulation.

Unestam and Gleason (*307*) reported that respiration of starved mycelia of *A. euteiches* was markedly stimulated by glucose, fructose, and acetate, but glutamate and leucine were only slightly stimulatory and butyrate had a negative effect. The respiratory quotient for endogenous respiration (less than 1) was increased

by glucose to values higher than the theoretical for complete oxidation of this substrate. Thus either this substrate suppressed endogenous respiration, or more likely oxidative assimilation of glucose had occurred.

Exocellular Enzyme Production

Cellulase

A cellulolytic enzyme, apparently a cellulase of the 'Cx' type, was detected in culture filtrates of several isolates of *A. euteiches* and in pea tissue infected with the fungus (*349*). The crude enzyme from culture filtrates was able to hydrolyze carboxymethylcellulose but not native cellulose. Enzyme activity was optimum at about pH 6. The disaccharide cellobiose was degraded by the enzyme preparations to glucose, but reducing sugars were not detected from carboxymethylcellulose. The degree of cellulolytic activity detected in culture filtrates of *A. euteiches* by Ayers et al. (*30*) depended on the isolate used. Partial purification of this enzyme was achieved by an ammonium sulfate fractionation procedure, which permitted a separation of the cellulase from a pectolytic enzyme that was also present (*30*).

Polygalacturonase

An exocellular pectolytic enzyme that may also be of importance in pathogenesis by *A. euteiches* is produced by the fungus in culture and in infected pea roots (*28–30, 349*). The enzyme was produced in cultures without pectic compounds, but greater amounts were produced in media containing glucose and small amounts of pectic substances (*29*). Pectolytic activity of extracts of infected tissue was similar in behavior to the activity of culture filtrates, in that sodium polypectate was hydrolyzed randomly to less viscous uronides without the release of appreciable amounts of galacturonic acid (*28, 349*).

The pectolytic enzyme of *A. euteiches*, apparently an endopolygalacturonase, was purified according to a procedure illustrated in figure 17. In the final purification step the enzyme was eluted from a column of Sephadex G-100 gel with 0.1 M sodium chloride. The enzyme emerged from the column in a single protein peak that was devoid of cellulase. The purified enzyme displayed the following properties (*30*):

(1) It hydrolyzed sodium polypectate and pectic acid more rapidly and completely than pectins; the rate of hydrolysis of pectins was dependent on their degree of methyl esterification.

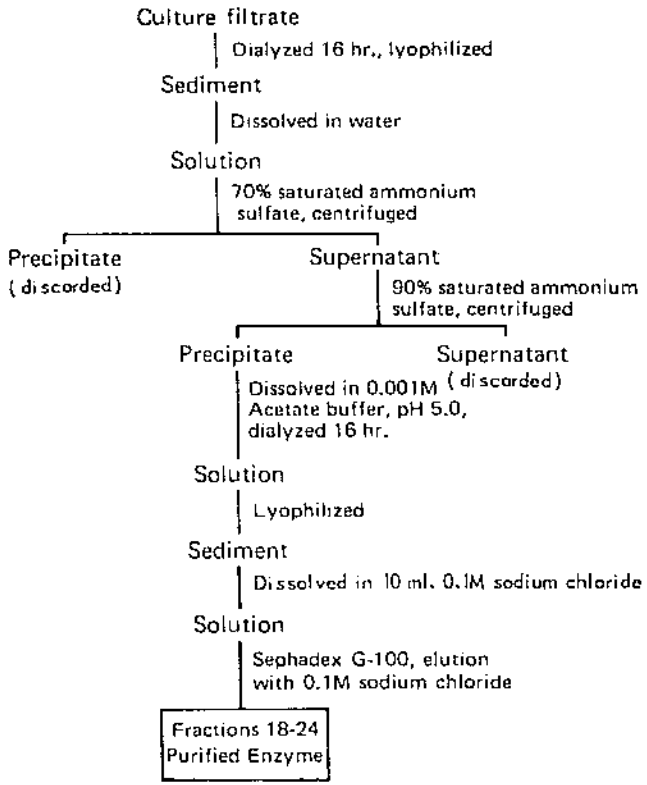

FIGURE 17.—Procedure for purification of polygalacturonase from cultures of *Aphanomyces euteiches*. (From Ayers et al. (*30*).)

(2) It released a series of oligomers of galacturonic acid as end products ranging from octogalacturonic to digalacturonic acid. Galacturonic acid was released only upon prolonged incubation.

(3) Its pH optima varied with the substrate used and shifted to lower values during substrate degradation.

(4) It softened and macerated plant tissue slices.

Although conclusive evidence was lacking, it was suggested that the endopolygalacturonase may function in the spread of *A. euteiches* within plant tissue during infection (*28*, *29*). The symptomology of infection, i.e., a slow development of water-soaked outer root tissue with discoloration and softening, was indicative of this type of enzymatic activity. Penetration of the root tissue by the fungus was postulated as a prerequisite, since the purified endopolygalacturonase had no macerating effect on intact, whole pea roots (*30*).

Reproduction

Zoospore Production

The medium used to cultivate mycelium for zoospore production, although important, does not seem to be critical. Several growth media were used to grow *A. euteiches* or *A. cochlioides* as a prelude to zoospore production. These included pea-seed decoction (*126, 160, 187, 280*), corn-kernel decoction (*187, 270, 275, 336, 342, 347, 348*), peptone-glucose broth (*218*), maltose-peptone broth (*187, 194, 338, 343*), potato-dextrose broth (*187*), 0.3-percent peptone or 0.3-percent soytone (*271*), peptone-dextrose salts (*202*), oatmeal agar (*126*), and cereals (*275*). Although synthetic media have not been used to any extent for massive production of asexual spores for experimental purposes, the glutamic acid-glucose-thioglycolic acid medium (table 3) permitted zoospore development and release soon after the medium was inoculated (*218*).

After a growth period that may vary according to the investigator from 2 to 14 days, zoospore induction is obtained by washing the mycelial mats with several changes of tapwater (*275, 348*), unspecified kind of water (*160, 289*), distilled water (*202*), changes of tapwater and distilled water (*187, 336, 348*), lake water (*218*), dilute solution of sodium chloride (*271, 348*), or dilute solution containing 1.75×10^{-4} M calcium chloride, 10^{-4} M potassium chloride, and 10^{-3} M magnesium sulfate adjusted to pH 6.5 (*218*).

In a study of the factors affecting zoospore production by *A. euteiches*, Llanos and Lockwood (*187*) found that the following conditions favored abundant zoospore production: (1) A mycelium of about 5 days old developed in maltose-peptone broth, (2) replacement of the medium with tapwater for 1 to 2 hours, (3) a second replacement of distilled water for 15 to 17 hours, and (4) a temperature of 24° C. Light had no effect on zoospore production. Forced aeration of the final replacement wash containing the mycelium increased zoospore production two to four times. Under these optimum conditions, zoospore concentrations of 2 to 3×10^5 per milliliter of the replacement liquid were obtained, although the numbers produced were variable because of inherent differences in individual isolates (*187*). Sherwood (*348*) found that two rinses of tapwater were superior as sporulating medium to distilled water or solutions of 0.002 M sodium chloride, magnesium sulfate, and sucrose.

Similar empirically determined conditions appear to favor zoospore production by *A. cochlioides*. Schneider (*264, 271*) found

that 5- to 7-day-old mycelial mats of *A. cochlioides*, which were developed in corn decoction or in 0.3-percent peptone, produced more zoospores than those 11 days old or older. The suspension of the mats in single rinses of tapwater at 20° to 25° C. for 16 hours permitted greater zoospore production than the use of distilled or demineralized water rinses. The optimum volume of replacement water was about three to four times the volume of the original culture medium. Sodium chloride at 0.002 M appeared to enhance asexual sporulation in either tapwater or distilled water. A pH range of 5.6 to 7.5 was satisfactory. As with *A. euteiches*, aeration of the replacement water further enhanced zoospore production by *A. cochlioides*. Upward of 1×10^5 zoospores per milliliter were obtained by this method (*271*).

Herr (*144*) investigated some of the factors that affect *A. cochlioides* zoospore production and motility. Of several complex media tested, 0.3-percent peptone was superior as the initial culture medium, and double deionized water gave more zoospores than distilled water or tapwater as the rinsing solution. Suspension of the rinsed mats in a solution of 1.75×10^{-3} M calcium chloride, 10^{-4} M potassium chloride, and 10^{-4} M magnesium sulfate at a pH of 8 for 16 to 18 hours at 24° C. gave the greatest yield of zoospores. Motility of collected zoospores was prolonged up to 48 hours at an optimum temperature between 16° and 20°.

The physiological significance of the empirical observations cited previously relating to zoospore production is just beginning to emerge. In a study by Mitchell and Yang (*218*) in which microcultures of *A. euteiches* were used to test factors affecting asexual sporulation, the following results were reported: (1) A factor in peptone promoted vegetative growth but prevented sporulation, (2) a sequence of washes of mycelium with lake water or with a solution containing salts of calcium, magnesium, and potassium promoted sporulation, and (3) there was an apparent loss of an endogenous factor(s) from the mycelium by the washing process, the absence of which promoted zoospore production.

The endogenous factor, although not directly detectable, appeared to be more abundant in aging mycelium than in young hyphae, and some evidence suggested that it was a volatile compound. Calcium ion was deemed essential for primary spore differentiation, whereas magnesium ion appeared to function in the development of mobility by the zoospores, and potassium ion seemed to stimulate germ tube development by secondary zoospores. Sodium ion in excess of 0.002 M was inhibitory to spore development and appeared to antagonize the calcium requirement.

A requirement for calcium for zoospore production and maximum motility of the zoospores by several isolates of *A. euteiches* was confirmed by Schoulties and Yang (*279*).

These findings suggest that tapwater or lake water rinsing of the vegetative mycelium furnishes ions essential for asexual differentiation and at the same time exerts a nutrient stress on the fungus by removal of organic nutrients diffusing from the mycelium. Loss of the hypothetical, volatile endogenous inhibitor of sporulation could also be expected to be hastened by this rinsing operation and by forced aeration, a condition that stimulates zoospore production in both *A. euteiches* (*184*) and *A. cochlioides* (*271*). Although Mitchell and Yang's autoinhibitor theory is attractive as an explanation for some of the observed results, the existence of such an inhibitor needs confirmation.

Zoospore Germination

Little information has been published on the organic or inorganic nutrients or specialized conditions required to germinate *Aphanomyces* zoospores. Undoubtedly before germ tube development can occur, the spore must already have, or accumulate from its environment, an energy source and nutritional building blocks necessary for synthesis of protoplasm and growth. Zoospores of *A. euteiches* germinate in dilute nutrient media, on root tissue, and even on water agar (G. C. Papavizas, unpub. observ.). However, it may be speculated that with water agar, traces of nutrients in the medium, as well as endogenous reserves within the cell, permit the development of the germ tube. The previous nutritional history of the zoospores also may be an important factor in determining whether germination occurs or not.

Mitchell and Yang (*218*) observed that, in addition to the inorganic requirements for calcium and magnesium ions for development of zoospores of *A. euteiches*, an exogenous supply of potassium ion appeared to stimulate germination of the secondary zoospore. Zinc ion had a similar effect. Thus it appears that zoospores may germinate in very dilute nutrient media including water and that certain exogenous factors may be required or at least stimulatory, but these have not yet been clearly defined. Without the presence of the nutritional factors listed previously or a living host root, the further development of the germ tube into a mycelium does not occur.

A. cochlioides zoospores derived from mycelial mats that had been washed six times in distilled water germinated to the extent

of 10 percent in the last rinse (*251*). Spot tests revealed that no sugars or amino acids were in the solution. The addition of various organic fractions of sugarbeet exudate stimulated zoospore germination in varying degrees up to a maximum of 82-percent germination for the crude exudate. The amino acid fraction, which was not further characterized, was the single, most effective stimulant. Approximately 50 percent of the zoospores germinated in this solution.

Oospore Production

Aphanomyces euteiches.—The development of sexual structures of *A. euteiches* was observed on complex media such as cornmeal agar (*160*), prune, malt, potato-sucrose, potato-dextrose, and starch-asparagine agars (*114*), all of which supported abundant oospore formation. Geach (*114*), however, observed that the fungus did not form oospores on cornmeal agar containing cornmeal, peptone, dextrose, and calcium carbonate, but it did develop oospores on plain cornmeal agar. The use of such undefined media, although adequate for morphological observations, provided little information on the nutritive factors relating to sexual reproduction.

Oospore development by three isolates of *A. euteiches* in a synthetic medium was first reported by Papavizas and Davey (*238*). This medium contained mineral salts, glucose, thioglycolic acid, and a mixture of amino acids added in proportions found in powdered yeast extract. A less complex medium, which had DL-glutamic acid substituted for the amino acid mixture, permitted oospore development by two of the isolates, but a third isolate failed to form oospores in the medium even though it was adequate for mycelial growth. This more nutritionally exacting isolate formed oospores abundantly, however, if thioglycolic acid was replaced by DL-methionine or L-cystine (*239*). The nutritional requirements for at least certain isolates of *A. euteiches* appear to be more exacting for sexual differentiation than for vegetative growth.

Conditions that favored oospore formation in synthetic media (*85, 239*) included the following: (1) A culture medium that satisfied the minimum requirements for vegetative growth, (2) a favorable balance of sugar carbon and amino nitrogen that was variable with the isolate, (3) a reduced form of sulfur, especially DL-methionine or L-cystine for certain isolates, and (4) an initial pH of the medium within the range of 4.9 to 5.4. Glucose and

fructose were the best carbon sources for sexual reproduction out of 17 carbohydrates and derivatives that supported some mycelial growth (*237*).

Certain culture medium ingredients appear to inhibit oospore development in growing cultures of *A. euteiches*. Geach (*114*) reported that peptone added to cornmeal agar prevented formation of oogonia and antheridia. Small amounts of sodium nitrate and ammonium sulfate in this medium had the same effect. Papavizas and Davey (*239*) noted mycelial growth but no sexual development in a medium of glucose, yeast extract, and casamino acids and in a synthetic medium containing relatively high levels of an amino acid mixture.

Mitchell and Yang (*218*) observed that peptone in a medium favored immediate vegetative growth of *A. euteiches* from a zoospore inoculum, but there was no evidence of oogonial initials. In the absence of peptone, oogonia were formed rapidly soon after the zoospores had germinated. In a similar study (*328*), certain peptide fractions isolated from peptone by a gel filtration technique were found to produce the same effect. Peptide fractions combined with glycine, arginine, methionine, and alanine permitted the differentiation of oogonia and the development of mature oospores, but when tyrosine and phenylalanine were also present, oogonia formed but failed to mature.

There is little definitive information about the physiology of oospore development that can be gleaned from studies of the pathogen in vivo. It is known that oospores of *A. euteiches* appear in pea root tissue as early as 24 hours following inoculation (*61*, *336*). The meristem region of the root tip is the first area of hyphal penetration and oospore development, and oospores are reported to become more abundant in this site than elsewhere. As might be expected, the initial inoculum level affects the rate and abundance of oospore production. A high inoculum level results in multiple infections and causes a more rapid progression of the disease than low inoculum levels. Accordingly the development of the sexual stage within the tissue is also progressively increased (*342*). The optimum temperature range for oospore formation, as well as infection of excised pea root tips by *A. euteiches*, is 20° to 25° C. (*61*).

Carlson (*336*) considered that oospore development occurred in vivo as a result of stress or adverse conditions. Nutrient stress, brought about by depletion of available nutrients for mycelial growth by the invading hyphae, was suggested as a triggering mechanism for oospore production. This concept, dating back to

work by Klebs (*169, 170*), has often been presented as an explanation for the onset of sporulation in fungi. However, further critical studies will be needed to establish its validity in this instance and to define the mechanism on the molecular level.

Aphanomyces cochlioides.—Oospore production by *A. cochlioides* was abundant in decoctions or homogenates of oatmeal, buckwheat groats, barley, and sugarbeet seedlings in an unpublished study by C. L. Schneider and D. L. Yoder (C. L. Schneider, pers. commun.). The synthetic media of Papavizas and Davey (*238*) and Yang and Schoulties (*329*) supported oospore production, as did peptone broth, soytone broth, and several other media. A nutrient concentration of about one-half the optimum for vegetative growth was optimum for oospore production. Schneider and Yoder found that transferring a mycelium developed in a nutrient medium to a solution of 10^{-4} M calcium nitrate, magnesium sulfate, and potassium phosphate, or to well water permitted abundant oospore development, whereas mycelium transferred to distilled water developed only a few oospores. The optimum pH for oospore production was from 6.3 to 6.7.

Oospore Germination

Aphanomyces euteiches.—Jones and Drechsler (*160*) observed that when mycelium from 15-day-old cornmeal agar cultures was transferred to hanging drops in Van Tieghem cells, many of the oospores germinated. If the transferred material was washed first to remove pieces of the medium, germination was indirect by differentiation of the developing germ tube to form zoospores. From unwashed material the oospores invariably germinated directly. The mode of germination was apparently dependent on the amount of nutrients present.

Studies of oospore germination have been hampered by a lack of methods for isolating oospores from mycelium. Yang (*326*) in a brief report has described a technique that utilizes sonication and isopycnic density centrifugation for separation of oospores of *A. euteiches* from vegetative mycelial mats. Bhalla and Mitchell (*39*) obtained oospores free of mycelium by passing mycelial mats through the digestive tract of water snails (*Helisoma* sp.). The snail excreta, which consisted almost entirely of oospores, were dispersed in water. Only a few of these oospores germinated on water agar disks in contact with pea roots within 5 days despite the fact that the inoculum was viable and capable of initiating pea root infection.

Yang (*327*) induced germination of *A. euteiches* oospores by treatment with the proteolytic enzymes chymotrypsin and pronase. Up to 50 percent of the oospores were induced to germinate by chymotrypsin at 250 enzyme units per milliliter. Both indirect and direct germination were observed by Yang (*327*), who believed that the mode of germination was related to the age of the oospore. Direct or hyphal germination seemed to be the dominant mode with oospores more than 4 weeks old.

Oospores within plant tissue or in soil debris particles may germinate much less readily than those developed in artificial culture media. Scharen (*261, 346*) obtained germination of 2 to 40 percent of the oospores of *A. euteiches* embedded in plant debris particles, in horse dung, and pea-sand leachates after 5 to 24 days of incubation. The oospores did not germinate in water. Oospores in soil also germinated within cellulose casings buried next to roots of peas, soybeans, beans, and corn. Apparently germination was greater next to pea roots than next to roots of other plants (40 percent for pea vs. 33 percent for beans, corn or soybeans). Sterile soil supported 16-percent germination and nonsterile soil 8 percent; thus the oospores seemed subject to the phenomenon of soil fungistasis. Semianaerobic conditions, plus some exogenous chemical activator, were thought to supply the stimulus for oospore germination (*346*).

Olofsson (*233*) investigated the effect of several chemical and physical pretreatments on oospore germination from pea roots infected with *A. euteiches*. Various amino acids, vitamins, sugars, and nucleic acids did not stimulate germination, nor did heating at 60° C., freezing, and alternate freezing and thawing. However, oospores were induced to germinate by adding hydrochloric acid to tapwater in which the root material carrying the oospores was suspended. At a pH range of 3.3 to 5.1, up to 40 percent of the oospores germinated. No germination was observed below or above this pH range. Olofsson (*233*) suggested that the pH effect might be indirect and that fungistatic substances in the plant that prevent germination may be inactivated by the acid reaction.

Aphanomyces cochlioides.—Oospore suspensions of this species were obtained largely free of mycelium grown in nutrient media by a blending technique (C. L. Schneider, pers. commun.). Less than 1 per 1,000 spores was observed to germinate in sugar-beet-soil leachate, beet seedling decoction, casein hydrolysate, and an inorganic salt solution. Exposure of oospores to temperatures of 0° to 50° C. did not affect the incidence of spore germination. The conditions favoring oospore germination by *A. cochlioides* are thus largely unknown.

HOST-PARASITE INTERACTIONS

Zoospore, the Infective Unit

It is apparent from the work of many who have used suspensions of zoospores as inocula in pathogenicity tests that zoospores of *A. euteiches* and *A. cochlioides* can incite root disease on their respective host (*126, 160, 194, 200, 264, 289, 342, 346–348*). The bulk of the available circumstantial evidence suggests that the mycelium of either species has a minor role in infection in natural soil. Scharen (*346*) presented evidence that oospores of *A. euteiches* provide the primary inoculum for new outbreaks of pea root rot. Many of these oospores did not germinate in soil, but those that did, especially those adjacent to plant roots, did so by means of zoosporangia and zoospores. Thus the primary infective unit seemed to be the zoospore.

Additional indirect evidence suggesting that the zoospore is the responsible propagule for disease initiation was supplied by McKeen (*200*), who observed that the specific conditions of temperature and moisture levels optimum for incidence and severity of sugarbeet blackroot in naturally infested soil coincided with conditions optimum for zoospore production by *A. cochlioides*.

Chemotaxis

Cunningham and Hagedorn (*82*) studied chemotaxis of *A. euteiches* zoospores in relation to excised roots of various plant species. Root segments from 10-day-old pea or corn plant cultured in tubes were placed in aqueous suspensions of motile zoospores. Within seconds, zoospores were attracted by the roots of both plant species. Within 1 hour there was a massing of zoospores most prominently in the region of elongation immediately behind the oldest part of the rootcap and at localized areas of the older parts of the root (fig. 18).

The chemotactic response of *A. euteiches* zoospores to the roots of nine pea cultivars of varying tolerance to pea root rot was apparently equal. Roots of eight other leguminous plant species also attracted zoospores. Cunningham (*338*) concluded that although the host plant did indeed actively attract zoospores of *A. euteiches*, there was no correlation of attractiveness with tolerance or susceptibility of a plant to infection by *A. euteiches*.

Motile zoospores of *A. cochlioides* are also attracted by host roots or by root exudates (*324*). Rai and Strobel (*251*) used glass capillaries filled with sugarbeet exudate and various chemical fractions of root exudate as model root tips to study chemotaxis. Zoospores

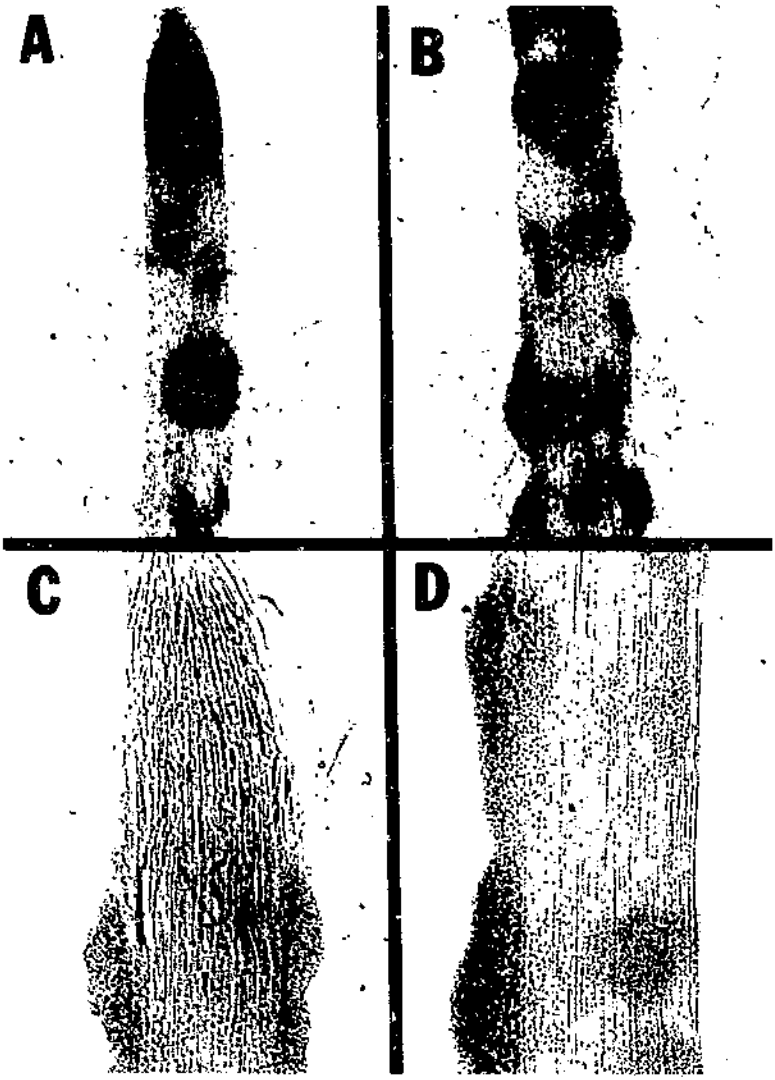

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FIGURE 18.—Roots of corn and pea 1 hour after being placed in a suspension of *Aphanomyces euteiches* zoospores: *A*, Root tip of corn; *B*, part of more mature root of corn; *C*, root tip of pea; *D*, part of pea root slightly back from the tip showing localized massing of zoospores. (From Cunningham and Hagedorn (*82*); courtesy of J. L. Cunningham.)

of *A. cochlioides* were strongly attracted to crude exudate, which also stimulated germination of approximately 80 percent of the massed zoospores. The organic acid fraction and the ion-exchange-

neutral fractions of beet root exudate were each considerably attractive to the zoospores, but neither stimulated germination. The amino acid fraction supported good germination and germ tube growth but did not attract the zoospores. Of several individual compounds tested, gluconic acid was the best attractant. Glucose and fructose, which were shown to be constituents of the exudate, were also good attractants.

The importance of chemotaxis in the disease cycle is still obscure. Haenseler (*126*) showed that zoospores of *A. euteiches* probably do not migrate in soil more than one-half inch at the most. Thus if chemotaxis has any role at all in infection, it must be of importance only in a very narrow zone surrounding the root. Porosity of the soil and moisture level, including the presence or absence of runoff water, however, may affect the migration of the zoospores and the radius of the chemotactic response. It may be argued that any means by which a fungus and host could be brought together, as by chemotaxis, would increase the chances of infection taking place. However, chemotaxis does not appear to be the mechanism of host-pathogen specificity in diseases incited by the *Aphanomyces* spp.

Invasion and Disease Development

Pea Root Rot

Cunningham and Hagedorn (*83*) observed the following sequence in the invasion of pea roots by zoospores of *A. euteiches*. First, the active zoospore came to rest on the surface of the root tip, encysted, and then germinated within 1½ hours by a simple germ tube. Within 2 hours most germ tubes entered directly between cells (fig. 19, *A*). Sometimes penetration occurred directly through the cell wall. Occasionally appressoriumlike structures appeared to be involved (fig. 19, *B*). Both the rootcap area and the area of root elongation were equally invaded.

After 8 hours, hyphae had penetrated as deeply as seven cell layers but were found more commonly in the first to third cell layers (fig. 20). Up to 12 hours many hyphae were intracellular, but after 61 hours almost all the hyphae in the thoroughly invaded cortex were intercellular. The phloem, pericycle, and stelar parenchyma were also invaded after 61 hours, and some disintegration of the outer cortex was apparent at this time.

A few oogonia were present after 61 hours in the inoculated seedlings studied by Cunningham and Hagedorn (*83*), but oospores appeared abundantly within 24 hours in the studies of invaded excised roots by Carlson (*336*) and by Cho and King (*61*).

The more rapid appearance of the sexual stage in the latter studies may be a reflection of the inoculum level used, since this affects the abundance and time of appearance of oospores (*336*).

Although Cunningham and Hagedorn (*83*) noted invasion of the stele in their studies, most of the available evidence suggests that the vascular cylinder is less vulnerable to attack and can remain as a functional water-transport system for some time, except in cases of severe infection or adverse environmental conditions

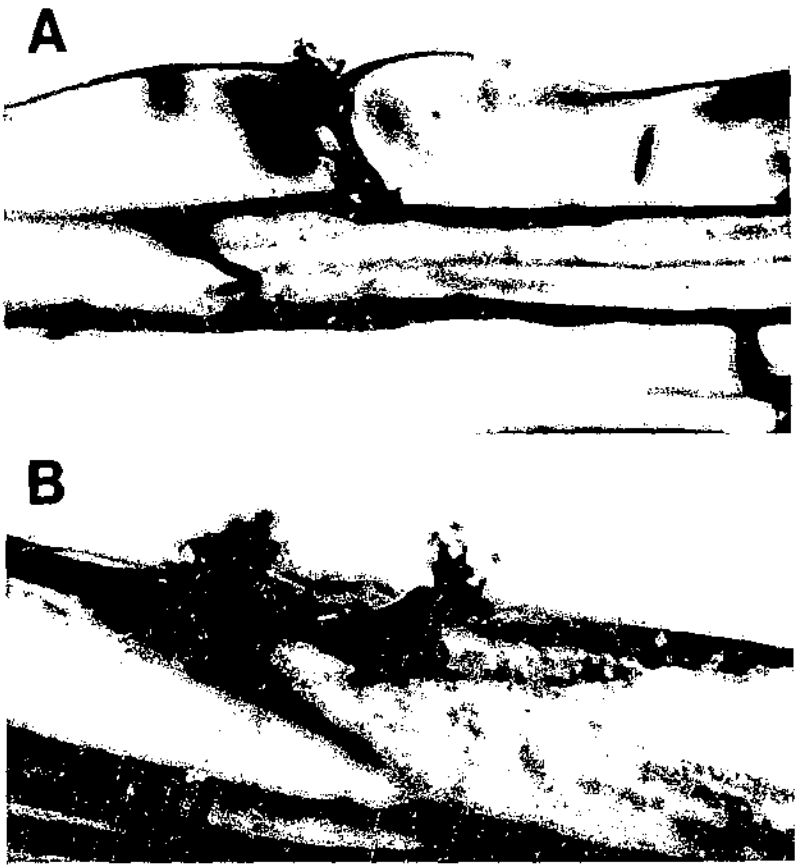

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FIGURE 19.—*A*, Longitudinal section showing *Aphanomyces euteiches* zoospores penetrating between adjacent epidermal cells of a pea root 2 hours after inoculation. *B*, Slightly tangential section of appressoriumlike structure only occasionally formed by germinating zoospores; it is fastened to outer wall of epidermal cell. (From Cunningham and Hagedorn (*83*); courtesy of J. L. Cunningham.)

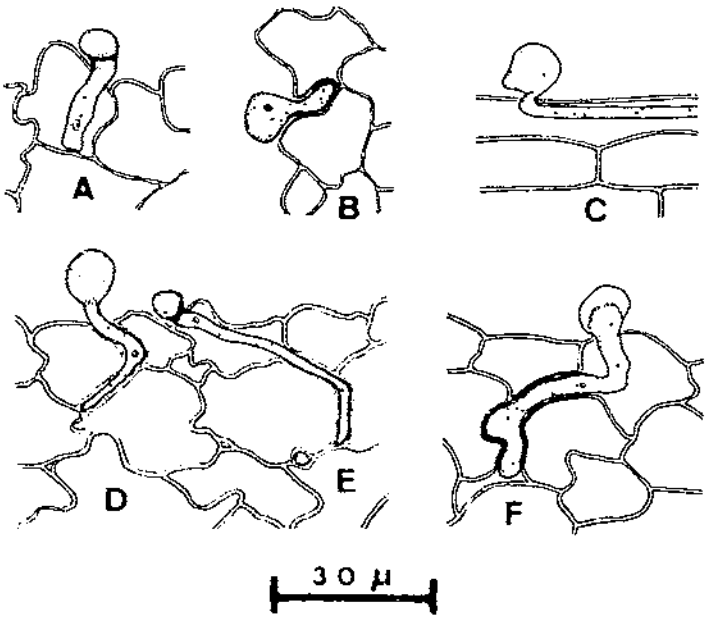

FIGURE 20.—Camera lucida drawing of penetration of pea roots by zoospores of *Aphanomyces euteiches* 8 hours after inoculation: *A*, *C*, *E*, and *F*, Penetration directly through epidermal cell wall; *B* and *D*, intercellular penetration. Wedging action of hyphae is clearly shown in *B*, *D*, *E*, and *F*. All figures were drawn from cross sections except *C*, which was from a longitudinal section. (From Cunningham and Hagedorn (*83*); courtesy of J. L. Cunningham.)

(*316*). Consequently the plants may frequently survive to maturity and give substantial crop yields even though the cortex of the underground parts of plants is decayed (*125, 126*). In severe root rot cases, the leaves shrivel, plants remain stunted, and they may eventually collapse and die.

The rapid intracellular and intercellular invasions of the root cortex observed by Cunningham and Hagedorn (*83*), as well as the resulting characteristic visible symptoms of water-soaked, softened areas on the root, are suggestive of an enzymatic hydrolysis of host tissue during invasion by the pathogen. The intercellular spread of the fungus from the infection site could be expected to proceed rapidly by the release of pectic enzymes, which would depolymerize the pectins of the middle lamella.

A polygalacturonase, active on both pectins and pectates, has been shown to be produced by *A. euteiches* in vitro and in pea root tissue (*28–30, 349*). This enzyme, purified from culture filtrates of *A. euteiches*, caused a perceptible softening and maceration of

healthy pea tissue slices in the study by Ayers et al. (*30*). However, since intact pea roots were not affected by the enzyme preparation, it was postulated that penetration of the epidermis of the pea root by the fungus must first occur before the enzymatic mechanism could be expected to have a role in pathogenesis.

Cellulase, known to be produced by *A. euteiches* in vivo as well as in vitro (*349*), may also be important during pathogenesis. The intracellular as well as intercellular penetration and spread of *A. euteiches* in pea root tissue suggests that a cellulase may be active in the breakdown of cellulosic cell wall material. Further critical histochemical studies for enzyme production in invaded plant tissue will be necessary to determine the importance of these hydrolytic enzymes, and possibly others, in the disease process.

Sugarbeet Blackroot

Less is known of the infection process of sugarbeet by *A. cochlioides* than of the pea infection by *A. euteiches*. There have been no recent histological studies of the step-by-step progress of the blackroot disease; however, McKeen (*200*) studied the development of the fungus in sections of infected sugarbeet seedlings.

Mycelium was found only in the intercellular spaces of the cortical tissue of the hypocotyl and root. The hyphae were rather scanty in affected tissue, and a relatively small part of the intercellular spaces was occupied. The fungus appeared to spread slightly in advance of visible symptoms. Cell walls adjacent to the mycelium became dark brown to black, and later in the infection process, considerable amounts of dark granular material surrounded the hyphae in the intercellular spaces.

McKeen (*200*) believed that the initial site of infection was through the hypocotyl at the soil line. He speculated that open stomata on the hypocotyl were the portals of entry of the germinating zoospores, since the sugarbeet hypocotyl has a thick cuticle and since the pathogen always appeared to be intercellular.

Two-hour exposure of roots to a zoospore inoculum of *A. cochlioides* is apparently sufficient for the initiation of infection (*202*), whereas the incubation period for the onset of visible symptoms of blackroot in infested soil is about 7 to 8 days (*146*). According to MacWithey (*202*), 4-day-old seedlings exposed to zoospores for 16 to 24 hours before being transplanted in soil exhibited symptoms of wilting and collapse of the hypocotyl tissue and typical symptoms of hypocotyl blackening. In contrast, seedlings exposed to zoospores for 2 to 8 hours showed only hypocotyl discoloration at the end of the eighth day. These differences in

disease expression were believed to be a function of the number of infections sustained by the seedlings.

Inoculum Potential

Many experimental studies of *Aphanomyces* spp. have been concerned with inoculum density, but because of widely differing methodology used by various investigators, it is difficult to make meaningful generalizations in the field situation. Although the oospore is generally recognized as the primary inoculum under natural conditions, most investigators have used suspensions of zoospores in experimental studies because (1) zoospores are easy to obtain free of other propagules, (2) they can be counted easily, and (3) they can be applied readily as uniform inoculum.

Studies With Zoospores

Johnson (*342*) applied zoospore suspensions of *A. euteiches* in varying concentrations to soil planted with peas. Maximum root rot developed when about 1.6×10^4 zoospores were applied per square inch of soil surface. Disease intensity was progressively less but not completely eliminated with inoculum densities of 4×10^3 to four zoospores per square inch.

Lockwood and Ballard (*194*) noted that an increase in *A. euteiches* zoospore concentration in aqueous suspensions, added to sand culture of peas, caused a corresponding increase in the disease index (fig. 21, *A*). About 1.5×10^3 zoospores per milliliter at 10 ml. per 10-inch row of pea seedlings were needed to obtain a consistently high level of disease. Similar results linking disease incidence and severity with zoospore concentration under a variety of experimental conditions have been reported by others (*35, 190, 202, 208, 264, 336, 341, 342*).

MacWithey (*202*) presented the following data, showing that the number of sugarbeet seedlings that developed hypocotyl infections after exposure for 24 hours to various concentrations of *A. cochlioides* zoospores was related to the zoospore concentration up to about 2×10^4 zoospores per milliliter.

Zoospores per milliliter (number)	*Hypocotyl infection (percent)*[1]
0	0
2×10^1	3
2×10^2	10
2×10^3	80
2×10^4	100
2×10^5	100

[1] Of 80 hypocotyls examined for each inoculum concentration.

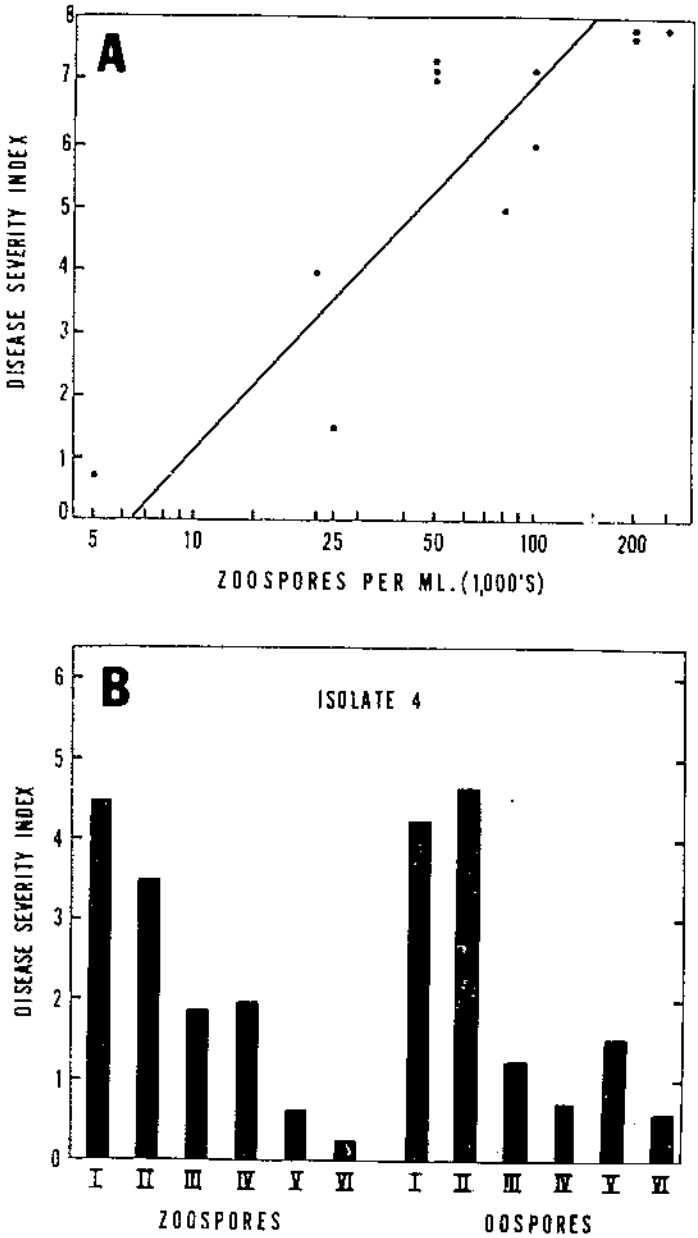

FIGURE 21.—*A*, Relation between disease severity index and concentration of zoospores of *Aphanomyces euteiches* used to inoculate 'Miragreen' pea seedlings (from Lockwood and Ballard (*194*)). *B*, Disease patterns on six pea cultivars produced by zoospore and oospore inocula: I, Miragreen; II, Early Perfection; III, P.I. 175232; IV, P.I. 169604; V, P.I. 180693; VI, P.I. 166159. Disease severity indexes produced by zoospores and oospores did not differ statistically at 5-percent level. (From Beute and Lockwood (*85*).)

Bhalla (*38*) attempted to determine the minimum number of *A. euteiches* zoospores required to initiate infection of pea roots. As few as three motile or two nonmotile zoospores per seedling were sufficient to initiate infection in 50 percent of pea seedling roots placed in polyethylene tubing (ED_{50}) at 28° C. In steamed soil and in nonsterile natural soil, ED_{50} values of 16 and 282 motile zoospores per plant were recorded, respectively. These low values indicated that a single zoospore could cause infection resulting in pea root rot in vitro. However, in natural soil the likelihood of infection from a single spore was reduced.

In further studies, Bhalla and Mitchell (J. E. Mitchell, pers. commun.) noted that infectivity based on ED_{50} values varied with the isolate of *A. euteiches* used. Temperature also had a pronounced effect on the numbers of zoospores required for initiation of infection. At temperatures lower than 28° C. the ED_{50} values increased progressively. It was not certain whether the effect of temperature was on the inoculum required for initiation of progressive infection or on symptom development.

The prevalence of blackroot disease among inoculated sugarbeet seedlings was reported (*203*) to increase logarithmically with the inoculum density of *A. cochlioides* zoospores. MacWithey (*203*) indicated that a certain unspecified minimum number of zoospores in an aggregate seemed to be required to induce typical symptoms.

Studies With Oospores

A direct relationship between inoculum density and disease incidence and severity has been observed with oospores as inocula. Boosalis and Scharen (*44*) screened plant debris fragments from soil and noted a correlation in the severity of root rot with the number of debris particles containing oospores of *A. euteiches*. Beute and Lockwood (*35*) compared oospore inoculum present in a naturally infested soil with zoospore inoculum, in inciting root rot in six cultivars of peas (fig. 21, *B*). The pattern of disease response with the two types of inocula was similar and indicated to these authors that zoospores could be used with confidence in assessing resistance of pea cultivars to *A. euteiches*.

The incidence of sugarbeet blackroot varied with the number of oospores per milligram of decomposing barley residues in the study by MacWithey (*204*). The addition of nitrate nitrogen to soil to decrease the carbon:nitrogen (C:N) ratio increased the oospore concentration in the residues. His results suggested that crop residues may increase the inoculum concentration of *A. cochlioides*, and the extent of the increase could be altered by

varying the C:N ratio. Schneider and Yoder (C. L. Schneider, pers. commun.) observed that sugarbeet seedlings developed typical blackroot symptoms when grown in 4-inch pots of sterilized soil infested with old, dried mycelium that was rich in oospores. Blackroot did not develop in pots infested with dried mycelium lacking oospores. About 7×10^1 oospores per pot were necessary to insure disease development. Later C. L. Schneider (pers. commun.) in another study noted an increased seedling disease severity with increasing dosages of the dried oospore inoculum.

A method for measuring the root rot potential of soils was developed by Mitchell et. al. (*214*). This method utilized screening of debris particles from soil followed by inoculation of pea seedlings in moist, rolled paper towels. It was useful for estimating the numbers or activity of propagules by their ability to cause infection. Although the kinds of propagules present and their absolute numbers could not be determined by the method, it allowed a soil with high root rot potential to be distinguished from a soil with low potential. About twofold differences in the amounts of inoculum could be detected.

Despite great attempts to understand the biology of oospores in the soil and rhizosphere, it is still not known what spore concentration in soil is the absolute minimum to initiate a progressive infection of *Aphanomyces* root diseases. The inability of many oospores in soil or debris particles to germinate at any one time under laboratory conditions contributes to our poor understanding of the dynamics of inoculum potential. Presumably a single germinating oospore within 1 cm. from the root may release sufficient numbers of zoospores from one sporangium to incite pea or sugarbeet root rot. However, the interrelated factors of moisture level and temperature, as well as unknown factors relating to the ability of the oospore to germinate, may be expected to affect disease development.

Mitchell et al. (J. E. Mitchell, pers. commun.) have calculated from their studies and from theoretical considerations of the space occupied in soil by an average pea root, that one root in four could be expected to contact a propagule in soil containing 1×10^2 oospores evenly distributed in 1 gm. of soil. Accordingly a concentration of 5×10^2 oospores per gram should permit approximately 100-percent infection.

VARIABILITY AND PHYSIOLOGIC SPECIALIZATION

Research on the variability and physiologic specialization of *Aphanomyces* spp. has been limited by the lack of resistant or

immune host cultivars. Strain differences of *A. euteiches* and *A. cochlioides*, however, have been reported. The following characteristics differ among isolates: Zoospore size (*35*), time required for sporulation and ability to produce zoospores (*166*, *336*), amount of growth in culture media (*85*, *237*, *238*, *341*) and in excised root tips (*336*), sexual reproduction (*85*, *237*, *238*, *336*), and production of pectinolytic and cellulolytic enzymes (*28*, *29*, *349*). No work has been done to separate strains on the basis of aversion (barrage) and rate of mutation.

Very little work has been done on physiologic specialization of *A. euteiches* and even less on that of *A. cochlioides*. King and Bissonette (*166*) were among the first to study physiologic specialization of *A. euteiches*. They found that six single-zoospore isolates from diseased peas collected from different parts of Minnesota differed in their ability to attack various cultivars and selections of canning peas. Two isolates were highly pathogenic, two moderately pathogenic, and the remaining two nonpathogenic on a common cultivar grown in Minnesota. Sherwood and Hagedorn (*287*) tested two different single-zoospore isolates of *A. euteiches* and found that only one parasitized *Lotus corniculatus*, *Onobrychia vicilifolia*, *Trifolium repens*, and *Vicia pannonica*. The second isolate infected only *Trifolium pratense* and *Amaranthus retroflexus*. Both isolates were pathogenic to peas.

Similar research of limited scope on the physiologic specialization of *A. euteiches* was reported by others (*190*, *259*, *336*). Carlson (*336*) used 10 isolates of *A. euteiches* obtained from diseased peas growing in Minnesota, New York, and Wisconsin and noted considerable strain differences in the ability to infect and produce oospores in excised root tips of tolerant and susceptible pea cultivars and of various wild and cultivated plants. Scharen (*261*) also noted differences in pathogenicity and cultural characters of seven single-zoospore isolates obtained from germinated oospores.

The most recent advances in the study of physiologic specialization of *A. euteiches* have been reported by Beute and Lockwood (*35*), Sundheim (*299*), Sundheim and Wiggen (*300*), and Carley (*335*). Beute and Lockwood (*35*) attempted to determine whether pathogenic races could be recognized on a series of pea cultivars and introductions that differed in their degree of resistance and susceptibility. Of 15 single-zoospore isolates of *A. euteiches* tested from various parts of the United States, 14 were similar in pathogenicity and designated as race 1. One isolate differed considerably in pathogenicity from the others; it was designated as race 2. Differences in pathogenicity of the two races on six pea cultivars and introductions are shown in figure 22.

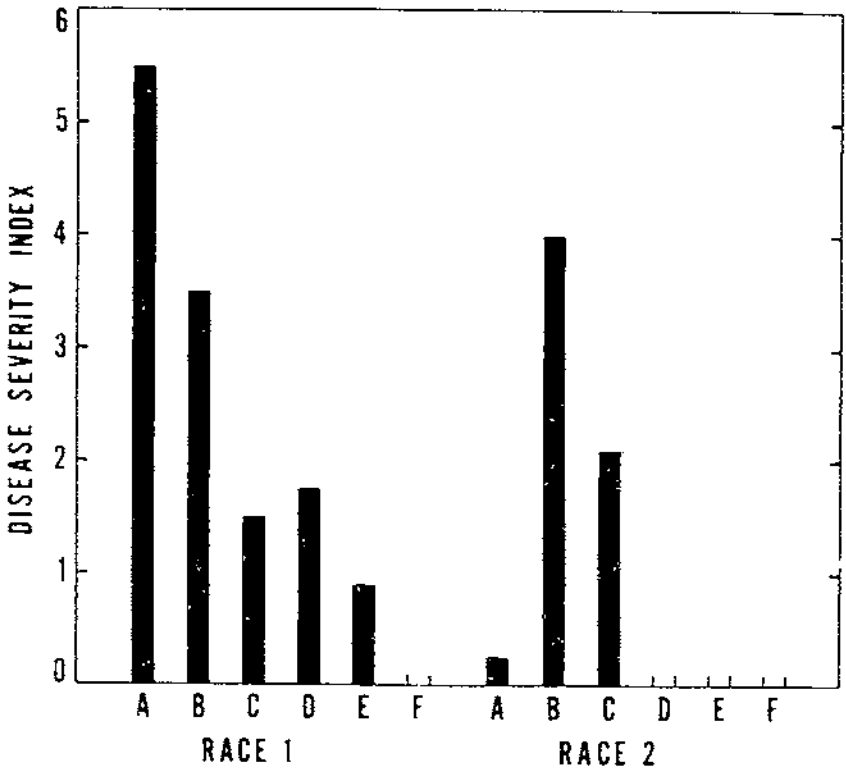

FIGURE 22.—Response of six pea cultivars and introductions to race 1 and race 2 of *Aphanomyces euteiches* (isolate 4 represents several isolates of race 1 and isolate 5 is race 2): *A*, Miragreen; *B*, Early Perfection; *C*, P.I. 175232; *D*, P.I. 169604; *E*, P.I. 180693; *F*, P.I. 166159. (From Beute and Lockwood (*35*).)

The degree and pattern of physiologic specialization were not altered by inoculum concentration or by the kind of inoculum used (zoospores or oospores). Neither the ability of an isolate to produce zoospores nor the time required for zoospore production could be correlated with the degree of pathogenicity.

Sundheim and Wiggen (*300*) experimented with 14 isolates obtained from Norwegian field soils and confirmed and extended for the first time the existence of physiologic races of *A. euteiches* in Europe. In their studies they used the same six differential pea cultivars used by Beute and Lockwood (*35*) and differentiated physiologic races on the basis of numbers of dead plants 10 days after inoculation of pea seedlings with zoospores. Sundheim and Wiggen (*300*) divided their 14 isolates into four physiologic races.

Their race 1 appeared to be identical with race 1 originally described in the United States (*35*). The remaining three races differed from race 2 of Beute and Lockwood and were designated by Sundheim and Wiggen as races 3, 4, and 5.

Carley (*59, 335*) devised a different method to distinguish physiologic races of *A. euteiches*. First, he showed that bean cultivars belonging to various *Phaseolus* species could be attacked by *A. euteiches*. Second, he was able to distinguish races among eight isolates tested on eight cultivars of *Phaseolus* species (table 5). Although Carley (*59*) suggested a workable method for identifying physiologic races of *A. euteiches*, much remains to be learned. In his preliminary experiments he (*59*) used only a small number of isolates of *A. euteiches* without standardized inoculum concentrations. Moreover, the susceptibility of his differential cultivars was highly variable and this variability is undoubtedly undesirable in studies of this nature.

TABLE 5.—*Detection and designation of* Aphanomyces euteiches *races on 8 cultivars of 3* Phaseolus *species* [1]

Phaseolus species and cultivar	Reaction with designated *Aphanomyces euteiches* isolate [2]							
	125	372	Mich-5	999	A4	CEM	NY-4	10
vulgaris								
Cherokee Wax	R	S	S	S	R	R	S	I
Contender	R	S	S	R	R	R	R	R
Round Pod Kidney	R	S	S	S	R	I	I	I
Topcrop	R	I	I	I	I	I	I	I
lunatus								
Florida Butter	R	R	R	R	R	R	R	R
Jackson Wonder	R	R	R	R	R	R	I	R
Sieva or Carolina Pole lima	R	S	S	R	R	R	R	I
coccineus								
Scarlet Runner	R	S	S	R	R	R	S	S
Race designation	1	2	2	3	4	5	6	7

[1] From Carley (*59*).

[2] R=resistant to *A. euteiches*, no visible root rot; S=susceptible to *A. euteiches*, visible root rot; I=susceptible to *A. euteiches*, sometimes difficult to observe root rot.

Downie (*339*) was unable to observe any differences in pathogenicity to sugarbeet and garden beet among 25 single-spore isolates of *A. cochlioides* in the greenhouse and 11 isolates in the field.

The geographical range of the isolates he studied, however, was limited to Minnesota. Buchholtz and Meredith (*50*) were the first to obtain some evidence concerning physiologic specialization in the chronic phase of the disease caused by *A. cochlioides*. In limited experiments they observed marked differences in the ability of six isolates of *A. cochlioides* to induce the chronic phase of blackroot of sugarbeets in the field. Two of their isolates were very pathogenic, two moderately pathogenic, and two nonpathogenic. Warren (*316*) also reported differences in pathogenicity on sugarbeet among 10 isolates in the greenhouse. McKeen (*200*) in Canada was unable to show any differences in pathogenicity among isolates and concluded that physiologic specialization may not be present.

Schneider (*347*) also found only minor differences in pathogenicity among some 40 single-zoospore isolates from Michigan, Minnesota, Montana, and Ohio. However, he observed differences among the isolates in their ability to cause the chronic phase on sugarbeets. Some isolates differed in rate of growth at 15°, 20°, and 25° C., whereas others differed only at 25°. Additional studies were performed by Schneider (*272*) on the extent of physiologic specialization of 10 isolates of *A. cochlioides* obtained from *Beta vulgaris, Spinacia oleracea,* and *Chenopodium album* grown in soils from five sugarbeet-growing areas of the United States. The moderately resistant cultivar U.S. 400 was susceptible to all isolates. No significant differences in pathogenicity were observed among the 10 isolates from the three hosts nor from the five locations.

DISEASE DEVELOPMENT IN THE GREENHOUSE AND FIELD

Inoculum Preparation

Zoospore Inoculum

Zoospores are the most satisfactory and most common form of inoculum of *A. euteiches* and *A. cochlioides* for greenhouse inoculations of peas and sugarbeets, respectively. Most of the techniques for zoospore production have been discussed previously. In our laboratory large quantities of zoospores are produced by the following procedure based on the methods of Llanos and Lockwood (*187*) and Schneider (*271*).

A. euteiches or *A. cochlioides* is grown for 4 days in 500-ml. Erlenmeyer flasks containing 100 ml. of sterile maltose-peptone broth (3 gm. of maltose and 1 gm. of peptone per liter). The broth

is decanted on the fourth day, 100 ml. of sterile tapwater are added to each flask, and the mycelial mats are allowed to stand in the tapwater. After 2 hours the tapwater is decanted and replaced with 200 ml. of sterile distilled water containing 120 mg. of sodium chloride per liter. The mycelial mats are aerated by bubbling air through the replacement distilled water for 10 to 12 hours at 20° to 24° C. Zoospore evacuation of the young thalli begins 6 to 7 hours after washing is complete. With this method, from 1×10^6 to 3×10^5 zoospores per milliliter may be produced. After the zoospore suspension is decanted for use as inoculum, water is again added to the flasks to obtain additional zoospores. This procedure may be repeated for several days. A few isolates of *A. euteiches* fail to produce zoospores with this method (*35*). However, these may do so if they stand in sterile distilled water for 48–72 hours before aeration.

Oospore Inoculum

C. L. Schneider (pers. commun.) developed special growth media for production of oospores of *A. cochlioides* to be used for field inoculations. Oospores were produced in abundance in various natural media, including oatmeal broth, cornmeal broth, sugarbeet leaves, cruciferous leaves, pearl barley, buckwheat groats, and lima bean broth. Homogenized oatmeal broth (5 gm. of oatmeal per liter) with a vermiculite carrier was the best medium for oospore production. *A. cochlioides* was grown in oatmeal broth adjusted to pH 6.6 after autoclaving. The oospore-containing culture was homogenized and mixed with vermiculite to dry. The dry inoculum could be stored and used when needed for greenhouse and field inoculation. Oospore inoculum progressively showed less infectivity after 1 year of storage at 4° C. as time increased. Nevertheless some oospores 3½ years old still incited infection of sugarbeet seedlings.

In our laboratory, oospore inoculum of *A. euteiches* for field inoculations is prepared by growing the fungus in an autoclaved cornmeal-sand mixture (sand 98 gm., cornmeal 2 gm., water 13 ml.) for 30 days and spreading the inoculum uniformly on the soil surface before disking.

Oospore inoculum in soil may also be used for field and greenhouse inoculations. This may be prepared by planting peas or sugarbeets in natural or pasteurized soil, inoculating the young plants with zoospores, allowing the disease to develop on the roots for 4 to 5 weeks under high soil moisture, removing the plant tops, and mixing the infected roots carrying oospores with soil (*137, 342*).

Oospore inoculum in roots was prepared by Pivaral (*345*) as follows: Peas were planted in autoclaved white sand in flats at 24° C. When plants were 10 cm. high, zoospores of *A. euteiches* were pipetted onto the roots. After 1 month's growth with heavy watering, roots were removed, washed, air-dried, and stored in polyethylene bags for later use.

Inoculation Methods

Greenhouse Methods

Various methods have been used to inoculate peas or sugarbeets with *A. euteiches* and *A. cochlioides*, respectively, in the greenhouse. Zoospore suspensions used as inoculum were applied to the surface of the soil after pea or sugarbeet plants were 5 to 8 cm. high (*166, 342, 347*). With *A. cochlioides*, 1×10^5 to 2×10^5 zoospores per 4-inch pot were needed to determine varietal resistance of sugarbeets.

Lockwood and Ballard (*194*) stressed the importance of carefully standardizing inoculation techniques for evaluating peas for root rot resistance. Pea seedlings were grown in sand without nutrients or in an autoclaved mixture of equal parts of ground pea and sand (*95, 194*). Pea seeds were surface disinfected and planted in rows 2 cm. deep in metal pans. The plants were inoculated once when 2 to 5 cm. tall by pipetting zoospores of *A. euteiches* adjacent to the rows, and the sand-peat mixture was saturated with water. After 3 to 4 weeks of growth, the plants were harvested and evaluated for disease severity. In Lockwood and Ballard's inoculation experiments, root rot development was affected by depth of planting and age of plants at inoculation, age of zoospores, concentration of zoospores and volume of suspension, and distance from the plant to the point where the inoculum was placed in the plant row.

The effects of nutrients on infection of peas by *A. euteiches* was studied by Papavizas and Davey (*242*) with the use of ½-gallon glazed crocks containing acid-washed white quartz sand (fig. 23). The sand was moistened with nutrient solution and three surface-disinfected pea seeds were sown in each crock. Each crock was covered with the bottom of a 7-cm. deep storage dish to reduce evaporation until seedlings emerged. Crocks were equipped with manometers to maintain liquids at desired levels. Immediately before inoculation the rubber sidearms of the manometers were lowered to drain off the liquid from the crocks, the sand was flushed three times with distilled water and three times with

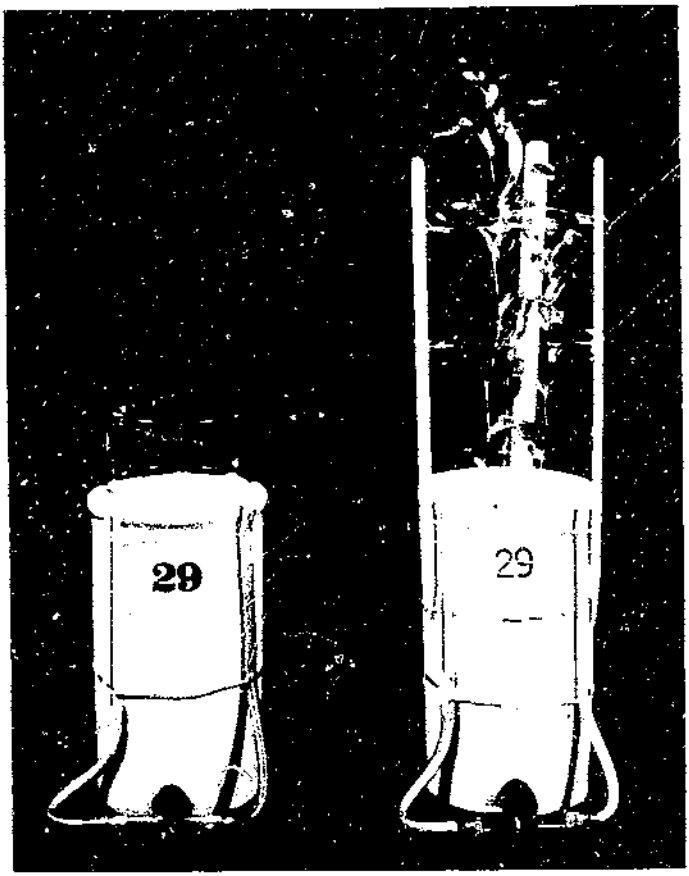

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FIGURE 23.—Glazed ½-gallon crocks containing washed quartz sand with manometers: *Left*, inverted storage dish bottom is placed on surface of sand to reduce excessive evaporation until seedling emergence is complete; *right*, pea plants supported by pot labels and strings. (From Papavizas and Davey (*242*).)

sulfate-free nutrient solution, and the rubber sidearms were returned to position. Zoospores (6×10^5 per crock) in nutrient solution were added to the crocks to within 0.5 cm. of the top of the sand. Subsequently the liquid level was maintained within 5 to 6 cm. of the top.

Additional special methods employing zoospores have been used to inoculate peas with *A. euteiches*. Haglund and King (*140*) grew pea seedlings for 7 days at 21° C. in sterilized vermiculite after which the root systems were washed in tapwater. The plants were

then suspended in a shallow tray containing an aqueous suspension of zoospores, incubated at room temperature for 24 hours, and transplanted into 5-ml. clay pots containing steamed soil. The uniformity and effectiveness of the inoculation method provided an accurate evaluation of varietal tolerance to common root rot.

The following method was used by Carlson (*336*) to inoculate excised root tips with *A. euteiches*. Lateral root tips from axenically grown plants were placed in a sterile Syracuse dish (52 mm. inside diameter) to which 5 ml. of zoospore inoculum had previously been added. After incubation for 24 hours at 20° C., the root tips were removed from the inoculum, placed in petri plates with 10 ml. of sterile distilled water, and incubated for 48 to 288 hours at 20° C.

Field Methods

Various methods have been used for field inoculation of peas and sugarbeets. Johnson (*342*) was among the first to use infested soil for field inoculations of peas with *A. euteiches*. In his experiments, row furrows were opened in the field, pea seed was planted, a measured volume of soil inoculum was placed over the seed, and the furrows were covered with field soil.

Schneider (*347*) compared the following three field inoculation methods: (1) Zoospore suspensions of *A. cochlioides* were applied to rows of emerging seedlings with a sprinkling can, (2) artificially infested soil was applied with the seed at planting with a fertilizer distributor, and (3) autoclaved sugarbeet seed, coated with vermiculite and nutrient broth and artificially infested with the fungus, was applied in the seed row with the fertilizer distributor. Field infections occurred only when the last method was used.

In subsequent experiments, Schneider (*267*) tested the following inoculated substrates in the field: (1) Infested steamed soil, (2) oat-grain medium, and (3) sorghum-grain medium. Inocula were applied in the drill row with a V-belt planter. All three methods of inoculation increased incidence of blackroot versus that in noninoculated plots. However, since each of the three media was tested by Schneider (*267*) separately at different times and in different locations, no direct comparisons can be made.

Methods of Disease Rating

Several methods of disease rating in peas infected with *A. euteiches* have been used (*35, 194, 285, 289, 342*). That developed by Smith and Walker (*289*) has been used perhaps more exten-

sively than any other method since the 1950's and is described here.

Plants are carefully lifted and examined for disease symptoms when 3 to 4 weeks old. Plants are individually rated on an arbitrary infection scale as follows (fig. 3): 0, no visible symptoms; 1, slight water-soaking on epicotyl or on primary or secondary roots; 2, moderate water-soaking on primary roots or epicotyl with light-brown areas confluent and more extensive but not involving entire root; 3, infected areas extensive, soft, but not collapsed, epicotyl not markedly shriveled; 4, extensive discoloration with tissue collapse and disintegration; dead plants are rated in this class.

A Disease Severity Index (DSI) is calculated by multiplying the number of individuals in each class by the class number, then multiplying the sum of the products of each class by 100, and dividing the figure by four times the total number of plants. The following is a simplified formula of calculation:

$$\text{DSI} = \frac{\Sigma(\text{disease class} \times \text{number of plants in that class})}{\text{total number of plants}} \times 25$$

With this system, when all plants are healthy, the DSI is 0, and when all plants show severe symptoms, the rating is 100. This method of disease rating has been used extensively (*84, 215–217, 235, 236, 244*).

Sherwood and Hagedorn (*285*) modified Smith and Walker's method to suit field conditions where large numbers of plants are usually rated. Pea roots are removed from the field at intervals of 40 to 70 feet and rated as follows: 0, no disease; 1, slight disease; 2, moderate disease; 3, severe disease. A field DSI is calculated by the formula—

$$\text{DSI} = \frac{\Sigma(\text{percent of total area in class} \times \text{class value})}{3}$$

A field DSI of 0 indicates that no root rot is in the field and 100 indicates that 100 percent of the field has severe root rot.

An indirect method of disease rating in peas involves determination of yield and tenderness (*285*). Readings are taken with a tenderometer and the data calculated as follows (*133*):

$$\text{Yield at tenderometer } 100 = \text{actual yield} - (\text{tenderometer reading} - 100) \times 28.7$$

If the tenderometer reading is greater than 100, the adjusted yield is lower than the actual yield and vice versa, because weight of peas increases with increase in maturity. A standard maturity is represented by a tenderometer reading of 100.

Disease ratings in sugarbeets infected with *A. cochlioides* are obtained by methods similar to those used in peas (*273*). Two to four weeks after inoculation each plant is assigned one of the following ratings according to the severity of blackroot (fig. 5): 0, no visible symptoms; 1, light symptoms; 2, moderate symptoms; 3, severe symptoms; 4, plants dead. A DSI for sugarbeets is calculated in a manner similar to that for peas (*289*).

Root Rot Potential of Field Soils

Since no quantitative techniques are available to determine inoculum density of *A. eutciches* and *A. cochlioides*, a greenhouse bioassay was developed to estimate root rot potential of field soils. The greenhouse technique for determining levels of soil infestation by *A. cochlioides* was first conceived by Fink (*109*) and developed by Fink and Buchholtz (*110*). The rationale behind this technique is the fact that it may be possible to predict how much root rot there is likely to be in a certain field by determining the inoculum potential of representative soil samples in the greenhouse by the susceptible plant assay method, provided conditions are favorable for root rot development.

Soil samples were taken from sugarbeet fields prior to planting and brought to the greenhouse. Treated sugarbeet seed was planted in these samples in 6-inch pots, and the percentage of seedlings infected with *A. cochlioides* in 30 days was determined and recorded for each sample. The degree or intensity of soil infestation in the field was deducted from the percentage of total seedlings infected in the greenhouse. In 1947 the correlation between intensity of soil infestation and estimated crop loss was 0.93, between intensity of infestation and percentage of deformed beets 0.73, and between intensity of infestation and weight of 300 beets −0.47.

Although Fink (*109*) and Fink and Buchholtz (*110*) suggested that greenhouse determinations of the DSI of field soils prior to planting offered considerable promise as a means of predicting sugarbeet losses in the field, no relevant continuation of this research can be found in the *A. cochlioides* literature. However, several papers on this subject appeared in the 1950's and 1960's on *A. eutciches* (*158, 232, 252, 253, 285, 342, 348*).

Johnson (*158, 342*) was the first to adapt the sugarbeet technique of Fink and Buchholtz (*110*) for peas. This technique is now used by some pea-canning companies (*348*). Johnson (*158*) indexed peafields for root rot potential by bringing to the greenhouse field soils representing the average topography of the field, growing peas in the field soil samples in pots under conditions optimum

for *A. euteiches* infection, grading the roots during blossomtime according to an arbitrary scale, and comparing the greenhouse DSI and field yields. A greenhouse DSI of 10–30 (low infestation) indicated that the field was safe for planting, 31–40 (moderate infestation) should be avoided if possible, and 41–100 (severe infestation) was hazardous to planting.

Reiling and King (*252*) determined the greenhouse DSI of soils from 49 fields in 1955 and 45 fields in 1956 in Minnesota and Wisconsin and compared the DSI values with the root rot that developed in the field. Later Reiling et al. (*253*) devised an elaborate soil indexing method for pea root rot caused by *A. euteiches* and *Fusarium solani* f. sp. *pisi*. Ten random samples were collected from a field and composited to provide 2 gallons of soil. After 30 to 40 days' growth in the greenhouse, the plants were harvested and severity of root rot was numerically rated as 1–2, clean roots; 2–3, light infection; 3–4, moderate infection; 4–5, severe rotting; and 6, death of infected plants.

Reiling et al. (*253*) found a highly significant correlation between the amount of root rot that developed in peas planted in soils in the greenhouse with the amount of root rot that subsequently developed in the fields from which these samples were taken (fig. 24).

These results with *A. euteiches* were substantiated by Olofsson (*282*) and by Sherwood and Hagedorn (*285*). The last two investigators distinguished three categories of fields on the basis of the greenhouse DSI: Safe for planting (index 0–50), questionable (index 51–69), and dangerous (index 70–100). They also observed that of all the factors responsible for yield differences in the field, variations in root rot accounted for about 49 percent of the total variation in the field. Avoiding "dangerous" fields appeared to be the only dependable method for pea root rot control.

Although field indexing has assisted growers as a stopgap method in avoiding losses from root rot, the method is subject to several difficulties. Lightly infested fields may give different results at different samplings during the same year. In these situations, environmental conditions during the growing season may be the most influential factor and make accurate predictions almost impossible. Although it is easy to induce and control root rot development in the greenhouse, it is not possible to do so in the field. Dry conditions in lightly or moderately infested fields may result in very little or no root rot; yet the field may have been kept fallow on the assumption that root rot would develop. Interference from other root pathogens such as *Pythium* spp., which can cause root browning and rotting, may also reduce the usefulness of the test.

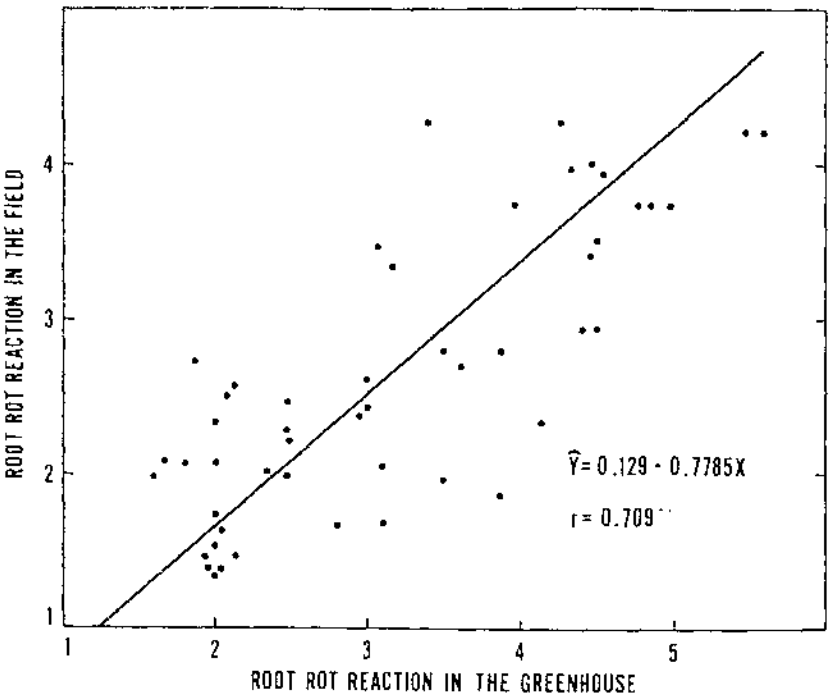


FIGURE 24.—Correlation between development of pea root rot caused by *Aphanomyces euteiches* in greenhouse tests and root rot development in 49 fields in 1955. (From Reiling et al. (*253*).)

In addition, samples from heterogeneously infested fields may sometimes overestimate field root rot potential.

Factors Affecting Disease

Temperature

The effect of soil temperature on the severity of common root rot of peas incited by *A. euteiches* was first studied by Jones and Drechsler (*160*). In their experiments, infection in the greenhouse occurred throughout the entire temperature range at which peas grow. Little disease developed at temperatures below 15° C. Haenseler (*127*) showed that *Aphanomyces* root rot was rarely found in New Jersey before the middle of May and that a minimum daily temperature of at least 14° lasting over several days was necessary for infection. In these earlier studies the temperature range at which root infection by *A. euteiches* could occur was between 15° and 34°, with an optimum somewhere between 15° and 30° (*125, 160, 161*).

The optimum range for pea root rot development in sand was reported by Smith and Walker (*289*) to be 24° to 28° C. No infection was obtained at 12°. Infection was slight at 16° and considerable at 32°. Similar results were obtained by Sherwood and Hagedorn (*285*).

Few recent studies can be found on temperature, but several on *A. euteiches* narrowed the optimum range for disease development between 20° and 25° C. (*61, 337, 348*). Temperatures for maximum disease development closely approximate those most favorable for growth of *A. euteiches* in pure culture (cf. section on Physiology of Causal Organisms).

More precise information about the role of temperature on disease development has been obtained by growing inoculated peas in sand under constant temperature or by exposing excised root tips to *Aphanomyces* zoospores in water. Root rot of peas developed well at 20°, 24°, and 28° C. sand temperature but was very mild at 16° (*194*). A maximum amount of disease was obtained at 25°, but this was not significantly different from that observed at 20°.

In another test, Lockwood (*190*) observed that root rot development of the pea cultivar Miragreen increased with temperature from 16° up to 24° C. With two plant introductions, however, root rot increased with increasing temperatures up to 28°. Differential resistance of the two plant introductions was expressed at all temperatures except 16° (fig. 25).

In excised root tip experiments of the cultivar Perfected Wales placed in zoospore suspensions and incubated for 24 hours at temperatures ranging from 5° to 40° C, no oospores developed in the root tip tissue at 5° and 40° (*61*). Oospore formation occurred at 25° and decreased at temperatures above or below that optimum (*61, 335, 337*).

More recently Burke and Mitchell (*53*) and Burke et al. (*54*) reported that infection of the taproot of peas was greater at 16° C. than at 24° or 28°, even though these temperatures are optimum for root rot development (*289*). Rot by pathogens other than *A. euteiches* was reduced by growing the seedlings at 16°. Infection due to *A. euteiches* at 16°, however, remained latent and symptoms seldom developed unless the plants were subjected to higher temperatures. In the experiments by Burke et al. (*54*) symptoms developed more rapidly at 28°, but by 18 to 21 days after planting, disease incidence was about the same in plants grown at temperatures ranging from 20° to 28° after an initial incubation at 16°.

These results would indicate that in soils where other root pathogens exist, there is an optimum temperature for infection at about 16° and an optimum temperature for symptom development at 28°.

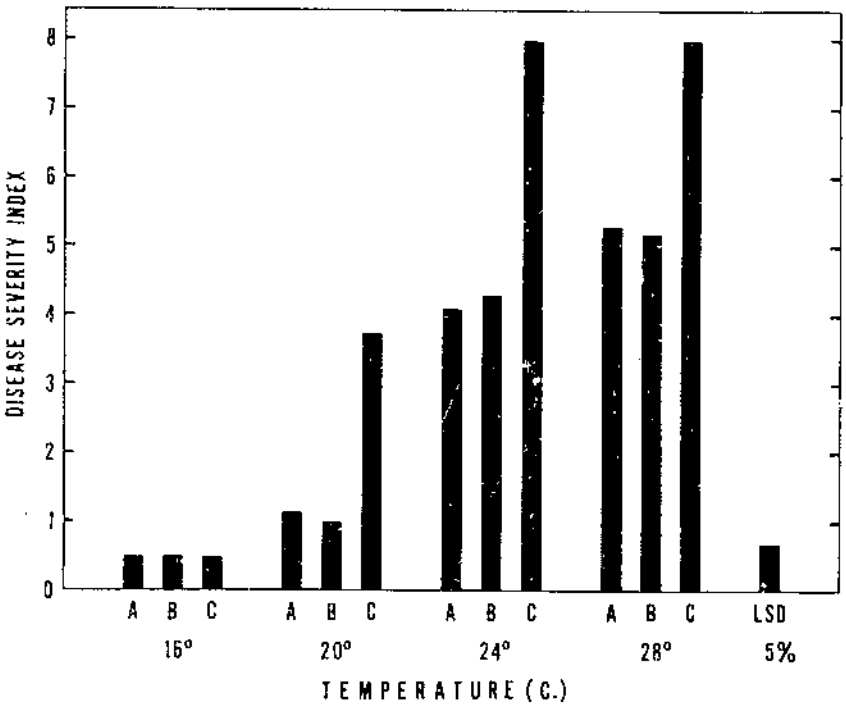


FIGURE 25.—Root rot severity of two pea introductions and cultivar Miragreen caused by *Aphanomyces euteiches* as affected by temperature: *A*, P.I. 180693; *B*, P.I. 169604; *C*, Miragreen. Least difference for statistical significance at 5-percent level was 0.7. (From Lockwood (*190*).)

Infection studies with *A. euteiches* in natural soil should then be performed at 16° rather than at higher temperatures. Also, peas planted early may escape *Aphanomyces* root rot to a greater extent than those of later plantings because more pea growth occurs before the threshhold temperature for symptom development is reached.

Data relating to the effect of temperature on the development of blackroot of sugarbeets incited by *A. cochlioides* are meager. At 13° C. and below there was only slight blackroot of sugarbeets (*200*). At 17°, blackroot became serious, and at 21° and 25° it was very severe. Similar observations had previously been made by Afanasiev (*7*).

Soil Moisture

The bulk of research on the effect of soil moisture has been done with *A. euteiches* of peas. This pathogen depends on high soil

moisture for infection, root rot development, and rapid spread. The favorable effect of high soil moisture on root rot development has been observed many times and serious disease outbreaks are exclusively associated with unusually wet seasons. When soil moisture reaches 30- to 35-percent saturation, severe pea root rot may occur (*289*), and frequent rains that maintain high soil moisture increase root rot severity (*161*). Reinking (*254*) and Reinking and Newhall (*256*) noted in their surveys that pea root rot was of relatively little importance during extremely dry growing seasons, even though the soil was infested with *A. euteiches*. Root rot was practically absent in New York in the dry year of 1939, widespread in 1940, a wet year, and not very common in the moderately dry year of 1941.

Root rot of peas is likely to be most severe in heavy, poorly drained, and compact soils or in soils in which water may be held by impervious subsoil or by subirrigation (*99, 160, 161, 186, 313*). The pathogen is also most persistent in such soils (*160*). Soils with excessive drainage, on the other hand, are not conducive to severe root rot, but their yield potential is too low to make them important for profitable pea culture (*160*).

Jones and Drechsler (*160*) reported that there was little difference in root rot severity whether peas were grown in the greenhouse in infested soil maintained at 30 and 60 or at 80 percent of the soil water-holding capacity (WHC). They noted, however, that disease severity in the field was greater in soil with a high moisture content or in soil in which water was held by impervious subsoil.

Haenseler (*126*) grew peas in soil at moisture contents ranging from 20 to 100 percent of WHC. He noted that root rot was favored by high soil moisture with 30 percent of WHC being close to a minimum for the disease. Even though 100 percent of the plants became infected when grown in soils with a moisture level of 30 percent and above, the effect of the disease on growth of the plant and subsequent yield was not very marked. Haenseler (*126*) also noted that no infection occurred at a soil moisture fluctuating between 40 and 20 percent. The highest infection of peas occurred when soils were kept consistently at 60 and 80 percent of WHC and at moistures fluctuating between 80 and 60 and between 80 and 40 percent.

Not all investigators agree on the minimum moisture content necessary for infection. Smith and Walker (*289*) reported that practically no root rot occurred when peas were grown in infested soil maintained at 45 percent of WHC, whereas 72 percent of the

plants became infected when grown in soil maintained at 75 percent of WHC. Smith and Walker's minimum is somewhat higher than that observed by Haenseler (*126*). Results similar to those of Smith and Walker were also reported by others (*114, 232, 311, 314*).

Burke et al. (*51*) recently studied interactions between temperature and soil moisture in relation to seedling infection in soil naturally infested with *A. euteiches*. Seven days after planting, all plants at 16° and 24° C. were infected by *A. euteiches* when the soil had been saturated for 24 hours before removal of plants. In nonsaturated soil maintained near field capacity, 56 percent of the plants were infected at 24° and 26 percent at 16°. The 16° soil temperature reduced root rot incidence in the nonsaturated soil but not in the saturated soil. Their results also show that only brief periods of soil saturation are needed for sufficient seedling infection by *A. euteiches*. Such brief periods of soil saturation may occur with several hours of rain, especially in heavy, compact soils.

Pea losses because of root rot may not necessarily be proportional to the amount of infection but may depend on the timing of infection in relation to the stage of pea growth and the amount and timing of soil moisture. According to Haenseler (*125*), if infection occurs early or infected plants are subjected to extreme drought at pod developing time, injuries may be so great that the entire crop may be lost. In a wet soil, and especially if infection occurs at later stages of plant growth, damages may not be appreciable as long as the plant water-conducting part is left intact by *A. euteiches* and provided other parasites or saprophytes do not invade the affected tissues.

Johnson (*342*), whose results essentially agree with those of Haenseler, noted that low soil moisture during the last half of the growing period was as detrimental to pea height and weight as a continuous low moisture throughout the growing period. Variation in soil moisture during the first and last half of the growing period in heavily infested soils affected plant growth more than it affected the degree of root rot.

There is practically no information on the exact moisture relationships of mycelial growth in the soil, oospore germination and zoospore production, and frequency of infection by the two *Aphanomyces* species. The fact that high soil moisture favors high root rot development may not be entirely due to increased oospore germination and zoospore release at high soil moisture. Information also is not available on the moisture relationships of oospore and zoospore survival in soil.

Soil Reaction

Very few reports in the literature concern the effects of soil reaction on the occurrence or severity of pea root rot or sugarbeet blackroot. Jones and Linford (*161*) were unable to detect any relationship between soil reaction and severity of pea root rot caused by *A. euteiches*. In our own greenhouse experiments we never failed to obtain infection and good disease development in soils of varying pH values (G. C. Papavizas, unpub. data). According to Haenseler (*127*), the optimum pH for infection was so near the optimum for pea growth that reactions capable of inhibiting infections by *A. euteiches* injured peas as much as or even more than the pathogen. Sulfur concentrations of 300 to 1,200 pounds per acre that reduced soil pH and disease were toxic to plants. His conclusions probably explain why there has been so little work done on this environmental factor.

Haenseler (*127*) stated: "It seems evident from these tests that there is little hope of reducing losses from root-rot by adjusting the soil reaction with lime or sulfur. Liming favors the growth of the peas but also encourages the disease. Sulfur, on the other hand, may reduce or prevent the disease but the quantity of sulfur necessary to prevent infection seems to injure the crop as much as or more than the disease itself."

Soil Type

There is a lack of agreement as to the effect of soil type on development of root rot. During the spring of 1924 Drechsler (*99*) conducted a survey of some pea-growing districts of Delaware, Maryland, and New Jersey to determine the prevalence of pea root rot. He noticed that the destruction of the cortex appeared to extend farther up the stem above the ground level on plants grown in loose, open soils than those grown in heavier compact soils. He also noticed that most of the fields in which plants were severely diseased were those containing a porous type of soil.

Jones and Drechsler (*160*) noted that in Wisconsin root rot appeared earlier and was more severe in red clay than in other types of soil. In Maryland they reported that peas grown in a very sandy soil were severely diseased. However, this field had an impervious subsoil, which greatly reduced drainage of water from the field. Jones and Drechsler (*160*) concluded that any soil that naturally retains water, or in which water is held because of its relation to impervious subsoils, provides the most favorable conditions for development of the disease.

In their pea disease survey conducted in 1924, Jones and Linford (*161*) examined fields consisting of 27 distinct soil types and seven groups of incompletely classified soils. No soil type was found that provided an environment in which root rot could not develop. With similar cropping histories, clays and clay loams had a higher percentage of severely infested fields than loams, silt loams, or lighter soils. In fields in which there was more than one soil type, disease usually appeared first in soils with the greater WHC or in poorly drained areas. Linford and Vaughan (*186*) also noted that pea root rot was more severe at first in heavy than in medium or light soils, but once established, root rot was remarkably persistent on all types.

In a greenhouse bed consisting of 10 cm. of a red loam overlying clay soil infested with *A. euteiches*, Geach (*114*) found root rot to be relatively mild after eight heavily watered pea crops. It was very severe when alluvial soil was used on top of the clay instead of red loam. Temp (*349*) noted that the soil type affected root rot development in peas. The greatest decline of root rot severity with time was observed in black silt loam and gray-brown silt loam, and the slowest decline in muck soils and in gray-yellow silt loams. Root rot potential decreased to a small extent only in fields with red clay soils.

Age of Plants

Schneider (*347*) demonstrated that the age of sugarbeet seedlings at time of inoculation with *A. cochlioides* affects blackroot severity. The older the seedlings were at the time of inoculation, the lower were the incidence and the amount of blackroot. In Schneider's experiments the greatest contrast between a resistant and a susceptible sugarbeet variety occurred when zoospore inoculum was applied to soil 15 days after planting. Both varieties appeared equally susceptible when inoculated 5 days after planting. Blackroot incidence was not great enough to demonstrate differences in its severity between the two varieties when they were inoculated 1 month after planting.

Similar results were obtained by Lockwood and Ballard (*194*), who obtained more pea root rot when 4- and 6-day-old seedlings were inoculated than when 8-day-old seedlings were inoculated with *A. euteiches*. In additional experiments with pea seedlings inoculated at 0, 8, and 21 days after planting, Lockwood (*190*) noted that root rot in the pea cultivar Miragreen and in two pea introductions tended to decrease with increasing age of plants at inoculation.

TB 1485 (1974) USDA TECHNICAL BULLETINS UPDATA

APHANOMYCES SPECIES AND THEIR ROOT DISEASES IN PEA AND SUGARBEET 2 OF 2

PAPAVIZAS, G. C. AYERS, W. A.

Age of Inoculum

Lockwood and Ballard (*194*) and Lockwood (*190*) showed that zoospores of *A. euteiches* from cultures grown for 4 days produced the highest amount of root rot in peas and those from 12-day-old cultures the lowest amount. Zoospores from 8-day-old cultures were intermediate in this respect. Lockwood and Ballard also observed that zoospores of *A. euteiches* 2 hours old (mostly motile) and 14 hours old (mostly encysted) produced the same amount of disease.

Location of Inoculum

Haenseler (*126*) studied migration of zoospores in natural soil by placing zoospore suspensions with a hypodermic needle at distances of one-half, 1, 2, and 3 inches from pea rows. No peas were infected when zoospores were applied at a distance of one-half inch or more from peas, whereas direct contact inocula resulted in 42-percent infection. Haenseler concluded that zoospores are unable to migrate any appreciable distances in soil and to produce saprophytic mycelium that might advance and infect peas away from the germinated zoospore. Lockwood and Ballard (*194*) obtained the highest amount of disease and least variation when zoospore inoculum was placed as close as possible to pea rows than when placed 2.5 or 5 cm. from the rows.

More recent experiments by Burke et al. (*51, 54*) with soils infested with several root rot pathogens of peas showed that the most effective arrangement for rapid root rot development by *A. euteiches* was when a layer of infested soil was sandwiched between two layers of vermiculite and placed only 1 cm. below the seed. On the other hand, peas grown in and extending beyond islands of infested soil implanted in noninfested field soil escaped severe root rot or root rot was effectively delayed. Infection by *A. euteiches*, which was most pronounced in the taproots between the growing point and the cotyledonary node, appeared to originate behind healthy root tips.

Interactions of *Aphanomyces* With Fungi, Nematodes, and Viruses

Fungi

Root rot of peas in the field is a complex disease incited by several fungal pathogens, including *A. euteiches* (*24, 254*), and possibly by nematodes (*138*). Blackroot of sugarbeets is also a complex disease incited by *A. cochlioides* and by various other pathogenic

fungi (*46, 77, 200*). Although plant pathologists generally realize that more than one pathogen is usually involved in the root rot complexes, it is very difficult to ascertain what role is taken by each pathogen and what is the nature of the interrelationships of the pathogens.

Buchholtz (*46*) studied the sequence of infection of sugarbeet seedlings by *Pythium debaryanum* and *A. cochlioides*. He noted that the effect of the latter began when seedlings had ceased dying from *P. debaryanum*, about 15 days after planting. Schneider (*347*), however, noted that *A. cochlioides* was able to infect sugarbeet seedlings, together with other fungi, during the very early stages of plant growth. No mention was made by Buchholtz (*46*) or by any other investigator of any interactions between *A. cochlioides* and other seedling pathogens.

Pivaral (*345*) in Wisconsin was unable to observe any interactions between *P. ultimum* or *P. debaryanum* and *A. euteiches* in the pea root rot syndrome. Since damping-off by *Pythium* spp. and *Aphanomyces* root rot are two of the important diseases of peas in Wisconsin and elsewhere, Alconero and Hagedorn (*25*) studied the effect of *Pythium* on *Aphanomyces* root rot. They noted that adding *P. ultimum* or *P. debaryanum* to soils infested with *A. euteiches* had very little effect on the incidence and severity of the total root rot.

From these results and the results of Cunningham and Hagedorn (*83*), who found that *A. euteiches* could infect pea root cells directly, Alconero and Hagedorn concluded that wounds made by *Pythium* did not appear to make the plants more susceptible to *A. euteiches*. Alconero and Hagedorn (*25*) also noted that the sequence of infection of peas by *Pythium* spp. and *A. euteiches* differed from that described by Buchholtz (*46*) for sugarbeets. *A. euteiches* was able to infect pea seedlings during the early stages of plant growth.

Nematodes

Although nematodes are known to affect the development and severity of root diseases of many plants (*250*), very few studies have been conducted on possible interactions between plant parasitic nematodes and *Aphanomyces* spp. Taylor et al. (*302*) reported that several species of parasitic nematodes were associated with roots of canning peas in Minnesota. However, adding large numbers of the spiral nematode (*Helicotylenchus microlobus* Perry in Perry, Darling & Thorne (=*pseudorobustus* (Steiner) Golden)) to soil infested with *A. euteiches* or *Rhizoctonia solani* Kühn did

not affect root rot severity (*301*). Although Haglund and King (*138*) noted the adverse effects of nematodes on pea yields, they did not establish a relationship between nematodes and *A. euteiches*.

Later, however, Haglund and King (*141*) showed that the nematode *Tylenchorhynchus martini* Fielding at populations as low as 373 nematodes per 5-inch pot increased *Aphanomyces* root rot considerably. The amount of root rot increase was related to the nematode concentration added to *Aphanomyces*-infested soil. In contrast, the presence of *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven did not appear to affect the development of *A. euteiches* on 'Little Marvel' peas despite the fact that the nematode increased faster in roots infected with the fungus than in healthy roots (*87*). Davis (*87*) even claimed that *Meloidogyne incognita acrita* Chitwood, in combination with *A. euteiches*, in some cases resulted in development of resistance to *A. euteiches*. Although Temp (*349*) and Temp and Hagedorn (*305*) reported that 18 plant parasitic nematode genera were found in *Aphanomyces*-infested soil brought to the greenhouse, they did not study possible interactions of nematodes with *A. euteiches*.

More recently Whitney and Doney (*321*) studied the effect of *Heterodera schachtii* Schmidt (sugarbeet nematode) in combination with *A. cochlioides* on sugarbeet yield. The main losses were caused by *A. cochlioides* alone. Whitney and Doney were unable to show any significant interactions between the nematode and *A. cochlioides* on yield losses. From a small trend indicating that greater losses were due to the complex than losses caused by each organism alone, they concluded that "small synergistic interactions between *H. schachtii* and *A. cochlioides* on sugarbeet do occur but are influenced by other factors."

Viruses

Viruses appear to have a significant role in the development of *Aphanomyces* root rot of peas. Farley and Lockwood (*108*) reported that three pea cultivars were more susceptible to *A. euteiches* and *Fusarium* (*Hypomyces*) *solani* App. & Wr. emend. Snyd. & Hans. f. sp. *pisi* (Jones) Snyd. & Hans. when plants were inoculated previously with any of the following viruses: Pea mosaic virus (PMV), bean yellow mosaic virus (BYMV), alfalfa mosaic virus (AMV), or pea enation mosaic virus (PEMV). A twofold increase in root rot severity was usually observed in the virus- and fungus-infected plants as compared with the fungus- or virus-infected plants.

More recent advances on the effect of viruses on root rot increases have been reported by Beute and Lockwood (*36, 37*), who studied this phenomenon in great detail. Their evidence suggested that infection with BYMV or PMV increased susceptibility of 'Miragreen' peas to root rot incited by *A. euteiches* or *F. solani* f. sp. *pisi* by increasing the exudation of nutrients from roots. Analysis of this problem was carried further by Beute and Lockwood, who found that pea roots of virus-infected plants exuded more amino acid, carbohydrates, organic acids, and nucleotides than did healthy roots. Increased exudation resulted in increased inoculum potential of the root-rotting organisms and, therefore, in enhanced pathogenesis. They also observed that the addition of root leachates from virus-infected plants to plants grown in plastic pots and inoculated with *A. euteiches* increased root rot more than did leachates from healthy plants. The root rot increase occurred only during the period of increased exudation from roots of virus-infected plants.

SURVIVAL AND DISSEMINATION

Although many areas of study have greatly increased our knowledge of the two most important species of the genus *Aphanomyces*, they have failed to explain satisfactorily the mechanisms of survival and decline of the two fungi in soil. The kinds of propagules that may carry *A. euteiches* and *A. cochlioides* from year to year over periods extending even up to 10 years, the sites of survival, the maximum longevity of the propagules, and the factors affecting survival have not been completely understood. Problems of techniques have mainly hampered studies on survival in natural microhabitats. Although numerous new techniques have been developed for studying root-infecting fungi in their soil environment, it still is not known how to recover oospores of *Aphanomyces* from soil free of plant debris or how to induce satisfactorily their germination in soil and in artificial media.

A. euteiches and *A. cochlioides* are soil invaders that can survive in soil for long periods by means of resistant spores (*164*). Jones and Drechsler (*160*) reported that *A. euteiches* may persist for at least 6 years after it has become established in the heavier soils of Wisconsin. Linford and Vaughan (*186*) cited evidence that *A. euteiches* can even survive for 10 years. Fifteen Wisconsin fields in which peas had *A. euteiches* root rot within 10 years from a survey (*159*) were found to contain *A. euteiches*. Jones and Linford (*161*) also noted that out of nine fields which had not been

planted to peas for 10 or more years, three were still thoroughly infested, whereas five were apparently free of the disease. Thus disease severity apparently decreases with time in the absence of peas, but the time required for a complete decline is exceedingly long. *A. euteiches* also seemed to persist longer in heavy wet soils than in soils less favorable for pea root rot development (*161*).

Longevity of Mycelium and Zoospores

Very little work has been done on the survival of *Aphanomyces* in soil. No one has been able to determine what happens to the mycelium of *A. euteiches* and *A. cochlioides* in the decomposing tissue in soil, and mycelium is universally considered to persist for short periods only. Mycelium of *A. euteiches* was readily lysed and destroyed by natural soil (*192*). This report and observations of Boosalis and Scharen (*44*) on the persistence of *A. euteiches* oospores in decayed pea residue and the lack of saprophytic ability of this organism in soil (*288*) support the view that *A. euteiches* and perhaps *A. cochlioides* do not survive in soil as mycelium.

In an attempt to determine whether mycelium of *A. euteiches* is capable of functioning as inoculum in soil, Mitchell et al. (*214*) performed a series of inoculation experiments of pea seedlings on moist paper towels. The rapidity of symptom appearance after inoculum application was used as a criterion to detect the kind of propagules of *A. euteiches* present in the inoculum. Seedlings inoculated with a disk cut from the periphery of an actively growing culture developed symptoms in less than 3 days. Symptoms of infections initiated by zoospores appeared in 3 to 5 days, whereas those initiated by oospores took longer. Since symptoms on seedlings on a towel inoculated with plant debris from infested soil became evident in about 3 days, Mitchell et al. (*214*) surmised that viable mycelium may be capable of functioning as inoculum in soil at some intervals of time after its incorporation into soil together with the colonized tissue. Since this information is incompatible with the generally accepted view that mycelium of *A. euteiches* is rapidly destroyed by lysis in the soil, more evidence is needed on the survival ability of mycelium in soil.

Zoospores also appear to have little survival value. Haenseler (*126*) studied the number of days that zoospores of *A. euteiches* remained motile and infective to peas in liquid culture. The number of active zoospores gradually decreased from 1 to 4 days until usually by the fifth day all zoospores were inactive. Infectivity of the zoospores was correlated with their motility. Thus infection

resulted from a 5-day-old zoospore inoculum but not from 6-day-old zoospores. Approximately the same period of viability was observed in soil (*127*). The question of longevity of zoospores must be pursued further not only with motile zoospores but also with encysted ones.

There is even more scarcity of information on the longevity of *A. cochlioides* zoospores. Schneider (*347*) used infectivity of inoculum expressed as numbers of seedlings surviving in soil 30 days after planting to determine zoospore longevity. He found that zoospores could survive in autoclaved and nonautoclaved soil for at least 10 days.

Longevity of Oospores

The probable importance of the sexual stage of *A. euteiches* and *A. cochlioides* in survival was suggested by several investigators. They estimated the importance of oospores by observing either the length of time during which infested soil remained infectious (*114, 127, 160, 342, 348*) or the duration during which growth and infection of fresh host tissue could still occur from colonized dead host tissue (*214, 348*). The assumption that *Aphanomyces* oospores remained viable in infested soil or in colonized dead tissue, which germinated to initiate growth or infection in vitro or to start new outbreaks of root rot in the field, should be considered circumstantial at best. Since, however, mycelium and zoospores appear to be incapable of prolonged survival, and root rots caused by *Aphanomyces* are known to recur in fields even after 5 or 6 years (*313*), or when 10 or more years elapse between outbreaks (*161*), even circumstantial evidence should be considered seriously.

Agar and liquid cultures of *A. euteiches* without oospores perished within a month (*114*), whereas liquid cultures with oospores remained viable even for 10 months at room temperature (*342*). Cultures of *A. euteiches* containing oospores on cornmeal agar (CMA) survived for at least 1½ years at 4° to 5° C., whereas those containing no oospores on CMA supplemented with sucrose remained viable for only 30 to 40 days (G. C. Papavizas, unpub. data). Eleven of fifteen cultures of *A. cochlioides* 7 to 7½ years old, grown on potato-dextrose agar or CMA and covered with mineral oil, remained viable at room temperature (*265*). Geach (*114*) also observed that *A. euteiches* persisted for at least 2 years in fallow soil kept free of all weeds, indicating that oospores may survive for at least that length of time in the field.

More direct evidence has been reported since 1958 that *A. euteiches* survives in soil for many years as oospores in the absence

of susceptible hosts. Boosalis and Scharen (*44*) observed oospores of *A. euteiches* in plant debris the season following an infected pea crop. Scharen (*261, 346*) was the first to germinate oospores found in decomposing debris and obtain infectious zoospores. Scharen concluded that *A. euteiches* survives in soil in the absence of susceptible plants by means of oospores embedded in organic debris. As the debris decomposes completely, the oospores will remain in a free condition in the soil matrix.

Oospores of *A. euteiches* were also shown to be viable and infectious after 2 years of alternate freezing and thawing or continuous freezing in dry, moist, and saturated sterile and natural soil (*348*). Only exposure to high moisture for 2 years reduced viability somewhat. Schneider and also Schneider and Yoder (C. L. Schneider, pers. commun.) observed that dried oospore inoculum of *A. cochlioides* progressively showed less infectivity at 4° C. as time of storage increased. Nevertheless oospores 3½ years old incited infection of sugarbeet seedlings. None of these investigators, however, studied maximum longevity of oospores under natural conditions.

Importance of Alternate Hosts in the Survival of *Aphanomyces*

Very little direct evidence can be found in the literature on the length of survival of *A. euteiches* and *A. cochlioides* in the field between host crops and on factors affecting survival. Oospores may carry the two species over from one year to the next, but little is known as to how *A. euteiches* and *A. cochlioides* persist for several years between successive host crops. Severe infections by *A. euteiches* occurred in fields where peas had not grown for 10 or more years (*127*). Also, *A. cochlioides* has been reported to occur in fields in which sugarbeets had never been grown (*339*).

Several workers (*185, 335, 336*) postulated that life of *A. euteiches* may be prolonged not only by the ability of oospores to remain viable in the soil for many years but also by the ability of the pathogen to parasitize many other plants. Although root damage of alternate hosts may be of minor importance, the two pathogens may live as weak facultative parasites producing new oospores in the infected roots. Development of new oospores in alternate hosts and release of the oospores in the soil matrix on the decomposition of the infected tissues may not only increase inoculum density but also release a fresh batch of viable oospores each year following infection of the alternate hosts.

Linford (*185*) first reported that *A. euteiches* may survive as a weak parasite on roots of apparently resistant cultivated plants

and weeds. Soon thereafter Haenseler (*127*) was able to infect with pure culture inoculum the legumes garden pea, Canada field pea, sweetpea, hairy vetch, cowpea, and sweetclover. He also observed mature oogonia and oospores of *A. euteiches* in the infected plants, though no aboveground symptoms from the infection could be noted in cowpeas and sweetclover. Haenseler also found oospores in Crimson clover, navy bean, red kidney bean, spinach, and timothy.

Studies by Geach (*114*), Sherwood and Hagedorn (*287*), and more recent studies in Minnesota (*61, 168, 335, 336*) established that numerous economic plants and weeds were susceptible to *A. euteiches* and that oospores developed abundantly especially in root tips. Carley (*335*) even postulated that the importance of alternate hosts in the survival of *A. euteiches* lies not only on the additional quantities of oospores added to the soil but also on the "continuous rejuvenation of the organism by passage through the alternate hosts." In all these studies, however, no direct proof was provided to show the actual importance of the alternate hosts in the survival of *A. euteiches* in field soils.

That the longevity of *A. euteiches* is increased by parasitization of alternate hosts in the absence of peas is supported, according to Carley (*335*), by the following reasons: (1) Crop rotations are not effective in reducing the incidence and severity of pea root rot (*186, 304*); (2) the pathogen does not grow saprophytically in soil (*287*); and (3) oospore survival cannot account for the longevity of the fungus in field soils (*185, 287*). Other crops in the cropping sequence that receive large amounts of nitrogen could serve as hosts for *A. euteiches*. Carley (*335*) found that the amount of root rot of corn caused by *A. euteiches* was increased by ammonium nitrogen. Application of ammonium nitrogen to corn would increase root rot and therefore add three times more inoculum to the soil than would nitrate nitrogen.

Downie (*339*) and Buchholtz (*47*) were among the first researchers to comment on the possible importance of alternate hosts in the survival of *A. cochlioides*. The susceptibility of pigweed and lambsquarters to *A. cochlioides* and the general distribution of these weeds in northern Iowa were thought to account for the presence of *A. cochlioides* in this area (*47*). Coons et al. (*72*) reported an increase in the incidence of blackroot of sugarbeets in soils that supported previously a dense stand of pigweed. When beets followed corn and soybeans, stands were significantly better than when sweetclover and a mixture of corn and sweetclover pre-

ceded sugarbeets. MacWithey (*202*) also reported that crop residues may increase inoculum concentration of *A. cochlioides* in soil. The extent of increase was determined by the carbon:nitrogen (C:N) ratio of the residue and could be altered by adding nitrate nitrogen to residue-soil mixtures of high C:N ratios. MacWithey, however, did not explain how crop residues increased inoculum concentration.

Not all the information available on *A. cochlioides* supports the contention that alternate hosts are essential to the survival and epidemiology of this pathogen. Schneider (*273*) cited the fact that in his studies and in studies of others (*47, 200*) no major crops grown in rotation with sugarbeets in the Great Lakes region of the United States became infected with *A. cochlioides*. Since in McKeen's experiments (*200*) only pigweed and lambsquarters were susceptible to *A. cochlioides*, McKeen concluded that weed hosts may not be necessary in building up the inoculum or aiding in survival. In his experiments, blackroot was only destructive in fields that had continuously or frequently been planted with sugarbeets.

The fact that Boosalis and Scharen (*44*) found viable oospores of *A. euteiches* in plant debris fragments from fields the season after a pea crop gives support to the thesis that alternate hosts may assist the survival of *A. euteiches* in the field. Before further conclusions can be drawn, however, the following points need considerable clarification: (1) Do oospores developing on alternate hosts actually carry the pathogen over from year to year for 10 years or more or do the alternate hosts assist survival by releasing a fresh supply of oospores annually? (2) How long do oospores survive in soil and what is their host range? (3) Is *Aphanomyces* capable of infecting plants other than the hosts and producing oospores?

Although considerable evidence exists to support the fact that *A. euteiches* and *A. cochlioides* produce oospores in roots of alternate hosts, care should be exercised in interpreting observations of this nature without full proof. Weimer (*317*) reported that oospores of *A. cochlioides* may be found in roots of vetch in the field in the Southern United States. Linford (*185*) claimed to have observed *A. euteiches* oospores in roots of alfalfa, sweetclover, and vetch growing in peafields infested with the pathogen. What Linford thought to be *A. euteiches* oospores in oats and barley roots, however, were identified by Drechsler (*102*) as belonging to *Aphanomyces cladogamus* Drechsler.

Infectivity of *Aphanomyces* Associated With Debris

It is apparent from previous sections on infection of alternate hosts and oospore production in infected tissues that a continuous renewal of oospore population may take place in soil even in the absence of peas or sugarbeets. Ultimately oospores produced in root tissue of plants will be liberated from the decomposing tissues and remain free in the soil matrix until they will either die or germinate and infect new plants. Depending on the time elapsed since freshly infected tissue is buried in soil and the time a new root grows in soil, the possibilities also remain that, in addition to oospores, mycelium and encysted zoospores may be viable in organic debris and capable of functioning as inoculum. The question that needs clarification in this section is whether propagules in organic debris are capable of initiating infection.

Although there is as yet far too little information available on the infectivity of propagules in organic debris, some evidence has been produced of the importance of this type of inoculum in root rot development. Kotila and Coons (*171*) were the first to use as inoculum the plant debris from one of their previous experiments in which *A. cochlioides* was present. After 46 days of growth in quartz sand, sugarbeets were equally diseased in sand inoculated with debris from diseased plants and in sand inoculated with pure cultures of *A. cochlioides*.

Boosalis and Scharen (*44*) were able to show not only that oospores of *A. euteiches* may be found in plant debris fragments in soil but also that a high positive correlation existed with the numbers of debris particles containing oospores and the severity of root rot in soil from which the plant debris fragments were separated. Scharen (*261*) also showed that most of the oospores in debris fragments were viable and germinated to produce zoospores (fig. 26). The percentage of oospores from debris that germinated ranged from 2 to 40.

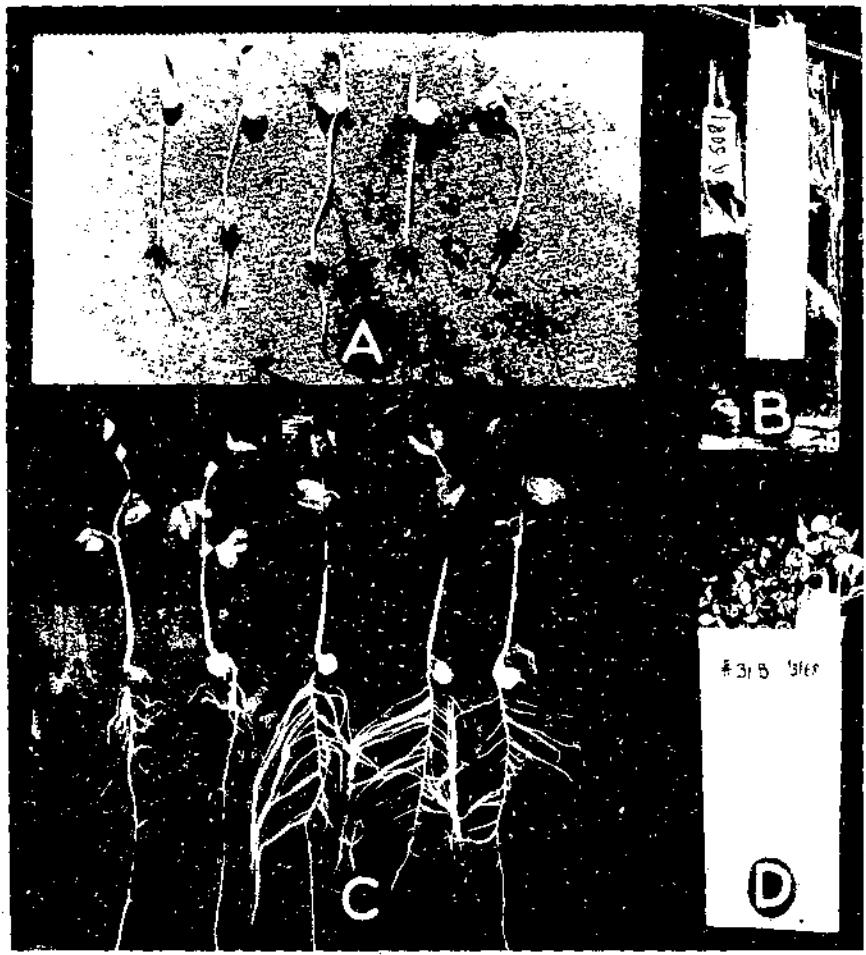
The most recent advances on the infectivity of inoculum associated with debris fragments have been reported by Mitchell et al. (*214*). To determine whether inoculum of *A. euteiches* in organic debris is infectious to peas, Mitchell et al. separated the debris from soil with wet sieving and tested the debris fractions for infectivity by a specially developed "rolled towel test" (fig. 27).

The largest proportion of infective propagules was observed in debris collected on a 200-mesh screen and in that which remained suspended in water for at least 10 seconds. The fractions differed not only in their infectivity but also in time required for symptom

expression. Inoculum in suspended debris produced symptoms on peas within the first week, whereas plants inoculated with inoculum in sedimented debris developed full symptoms in 3 weeks. By using this method, Mitchell et al. (*214*) were able not only to detect *A. euteiches* in organic debris from soil samples and estimate numbers or activity of propagules of the pathogen but also to show conclusively that propagules in organic debris are highly infectious to peas.

PN-3593

FIGURE 26.—Oospores of *Aphanomyces euteiches* in plant debris. Two oospores germinated (× 500). (From Scharen (*261*); courtesy of A. L. Scharen.)



PN-3504

FIGURE 27.—The Wisconsin "rolled towel test" for *Aphanomyces euteiches* for testing infectivity of organic debris: *A*, Inoculated pea seedlings; *B*, inoculated seedlings in roll ready to go into labeled plastic bag; *C*, seedlings at time of first reading; *D*, test rolls in aluminum box. (From Mitchell et al. (*214*); courtesy of J. E. Mitchell.)

Competitive Saprophytic Ability

There is a great scarcity of information on saprophytic growth of *A. euteiches* and *A. cochlioides* in soil independent of the host and on their ability to colonize dead organic substrates in soil. When autoclaved soil was inoculated with disks from potato-dextrose agar (PDA) cultures of *A. euteiches*, the fungus grew

rapidly through the soil from the inoculum food base of soil moistures of 100- and 160-percent field capacity and somewhat slower at 66-percent field capacity (*285, 287*). *A. euteiches* could not be recovered from autoclaved soil inoculated with naturally infested soil or from nonautoclaved soil seeded with PDA cultures, cultures on autoclaved pea roots, or naturally infested soil. In axenic cultures, mycelium and oospores formed abundantly in fumigated pea tissue, sparsely in winter wheat, oats, and sweet corn, and not at all in rye and barley (*287*). Winner (*323*), who was unable to obtain growth of *A. cochlioides* in nonsterile soil, concluded that saprophytic growth of this pathogen is of no importance for plant infection under natural conditions.

From the limited amount of evidence discussed and from unpublished information (G. C. Papavizas, unpub. data) on the extreme sensitivity of *A. euteiches* to antimicrobial agents, it may be possible to conclude that competitive saprophytic ability may not have an important role in the survival of *A. euteiches* and *A. cochlioides* in soil. More critical studies are needed, however, to establish whether the two species are soil-inhabiting or root-inhabiting fungi.

Dissemination

Aphanomyces spp. are not expected to be disseminated by movement of free oospores or zoospores through the air. Oospores are found in the soil or firmly embedded in organic debris. Zoospores produced under high soil moisture are also found in the soil. Zoospores, however, do not survive dry conditions for long periods. Jones and Drechsler (*160*) reported that *A. euteiches* could be carried from field to field on infected host plants or in infested soil.

Inoculations of new fields with the pea nodule-producing bacterium, for instance, may be responsible for dissemination. Soil from an old peafield in Maryland in which peas were diseased with *A. euteiches* root rot was used to inoculate new fields. Peas became infected with *A. euteiches* uniformly throughout each of the inoculated fields. In another case, where *A. euteiches* severely damaged a field where peas had not been grown for several years, Drechsler (*99*) observed that the field had been fertilized with pea vines from another field.

Dissemination may also be achieved when dry soil and debris from infested fields are blown by wind and carried onto neighboring peafields. Very little experimental evidence has been produced, however, to substantiate without any doubt this mode of dissemination. Jones and Drechsler (*160*) believed that the area of

the Truax Prairies in Eau Claire County of Wisconsin became infested with *A. euteiches* in this manner. A similar situation was thought to have occurred in Rochelle, Ill. Haenseler (*127*) found that *A. euteiches* survived in air-dried soil for up to 6 weeks, the maximum time tested, and still caused disease when the soil was planted to peas. He concluded that wind carrying light sandy loam and debris containing viable oospores could be transported from field to field in New Jersey.

The flow of surface water from neighboring fields was also cited by Jones and Drechsler (*160*) to be another means by which *A. euteiches* could be introduced into a new field. Tools, agricultural machinery, and humans may also be responsible for disseminating *A. euteiches* and *A. cochlioides*.

HOST AND PATHOGEN NUTRITION IN RELATION TO ROOT ROT DEVELOPMENT

Effect of Inorganic Nutrients

Although a considerable number of publications in the late 1920's and the early 1930's suggested the use of fertilizers to reduce *Aphanomyces* root rot of peas or blackroot of sugarbeets, the first substantial evidence that nutrition of the host or pathogen or both may affect root rot development and expression came from the University of Wisconsin. In those studies prior to 1940, nitrogen appeared to be the most important compound affecting pea root rot. Phosphorus and potassium were considered less important. Complete fertilizers with nitrogen applied before infection occurred reduced root rot in the field more than those without nitrogen (*314*).

Smith and Walker (*289*) were the first to study the effect of nutrition and ion balance in relation to pea root rot development. Nutrient solutions of varying composition and strength were allowed to drip at a constant rate into containers filled with washed, sterilized silica sand. With dilute nutrient solutions, they found that development and severity of *Aphanomyces* root rot were independent of the nutrient-ion balance and of the form of nitrogen. Since their low concentration experiments failed to show any effect of the form of nitrogen on root rot development, Smith and Walker surmised that the beneficial effect of mineral fertilizers containing nitrogen was dependent on factors other than nutrient-ion balance alone. When the nutrient solution was used at levels up to five times the normal strength of the solution described by Hoagland and Snyder (*153*), infection and disease severity decreased in direct proportion to the concentration of the nutrient

solution. Variations in the amount of nitrogen from 0 to 15 percent more than in the normal solution had little or no effect on the degree of root rot.

Smith and Walker were unable to detect any effect on disease development, whether in dilute or concentrated nutrient solution, when they varied the ratio of each of the elements nitrogen, phosphorus, and potassium from complete absence to an excess of that in the balanced solution. They concluded that a high osmotic value of the nutrient solution was responsible for disease reduction rather than the presence or absence of specific nutrients. It also seemed probable to them that the ameliorating action of the nitrogen-bearing mineral fertilizers in the field is due to the greater activity of the nitrogen present in the fertilizer in increasing the osmotic value of the soil solution rather than to its nutritional value.

The results of Smith and Walker (*289*) appear to contradict earlier work (*314*), which showed that increased levels of nitrogen were more effective than increased levels of either phosphorus or potassium in reducing pea root rot severity. To explain this contradiction, Smith and Walker (*289*) cited the work of White and Ross (*320*), who found that the nitrogen compounds sodium nitrate, ammonium sulfate, and ammonium nitrate increased the salt concentration of soil solution much more than corresponding quantities of the phosphorus and potassium salts used in commercial fertilizers. The possible inhibitory effect of nitrogen on the growth of *A. euteiches* observed by Geach (*114*) in his experiments with high concentrations of nitrogen sources was also ruled out by Smith and Walker (*289*), because root rot development was equally inhibited in solutions of high salt concentrations whether nitrogen was present in excess or lacking entirely.

Now other lines of evidence suggest that osmotic concentration of the soil solution may not be the only explanation for *Aphanomyces* root rot reduction by simple or complex mineral fertilizers. Wade (*311*) obtained considerable reduction of the pea root rot by low applications of potassium chloride to a soil with a low salt content. In waterlogged pots it almost eliminated plant death due to root rot, and although most of the plants became infected, root rot symptoms were less severe. In this case, a nutrient effect appears to be a more probable explanation of the response than an osmotic effect, especially since the soil used was very low in salts. Nutrition by minerals other than nitrogen affected development of blackroot of sugarbeets (*171, 324*). Necrosis of hypocotyls and root tips was enhanced in phosphorus-deficient plants. Phosphate added in the seedbeds reduced damage by early *A. cochlioides* infection.

To provide some information on this general question, Afanasiev and Carlson (*15*) studied the effects of phosphorus:nitrogen (P:N) ratios on sugarbeet development and blackroot severity. Their results emphasized the importance of balanced fertilization in seedling disease development. The amount of disease was less with nitrate than with ammonium nitrogen. When manure was added to mineral fertilizers, the amount of disease was very low in all P:N ratios (fig. 28).

The P:N ratios also appeared to affect the amount of disease. Sugarbeets were more susceptible to disease when grown in soil deficient in phosphorus or nitrogen or both or in soil with an unbalanced P:N ratio. There can be no doubt from these results that the concept of osmotic concentration may not sufficiently explain the increased resistance of sugarbeets to blackroot as a result of increased concentration of mineral nutrients.

From the evidence discussed thus far, nitrogen appears to have a very important role in *Aphanomyces* root rot development, irrespective of the mechanisms involved. Most workers agree that

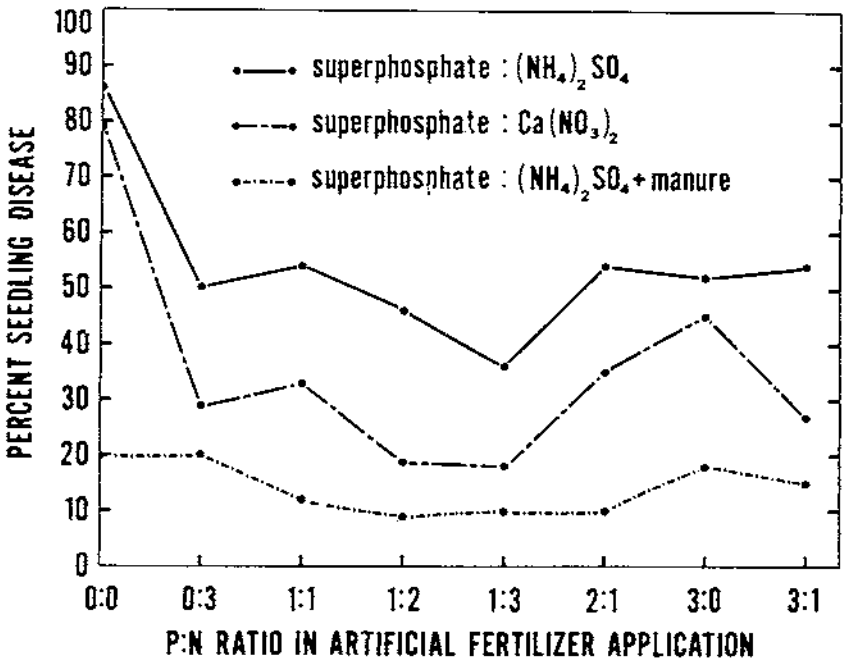


FIGURE 28.—Effect of phosphorus:nitrogen (P:N) ratio in artificial fertilizer applications of ammonium sulfate, calcium nitrate, and manure on seedling disease of sugarbeets incited by *Aphanomyces cochlioides*. (From Afanasiev and Carlson (*15*).)

several simple nitrogen sources and commercial fertilizers containing nitrogen may reduce root rot of peas or blackroot of sugarbeets. However, there is considerable disagreement on which of the two forms of nitrogen is more effective. Smith and Walker (*289*), who performed their experiments in sterilized, washed sand, could not detect any difference between ammonium and nitrate nitrogen in their ability to reduce pea root rot. Afanasiev and Carlson (*15*), however, found nitrate nitrogen to be more effective against blackroot of sugarbeets than ammonium nitrogen.

Carley (*335*) and Carley and King (*60*) conducted extensive experiments to determine the effect of nitrogen forms in nutrient solutions on *Aphanomyces* pea root rot severity. Ammonium nitrogen added as ammonium sulfate increased root rot, whereas nitrate nitrogen supplied as calcium nitrate had either no effect or suppressed root rot. Field studies by Carley (*335*), however, showed only a general trend in which nitrate nitrogen decreased and ammonium nitrogen increased root rot. Nitrogen per se had no effect on root rot severity in autoclaved soil. Carley (*335*) attributed the decrease of root rot by nitrate nitrogen to an increase of micro-organisms antagonistic to *A. euteiches* by nitrate but not by ammonium nitrogen. Under an ammonium nitrogen regime, little inoculum of *A. euteiches* was required for symptom expression, whereas with nitrate nitrogen a large amount of inoculum was needed to obtain an equal amount of root rot.

In contrast, Papavizas and Lewis (*244*), working with soils naturally infested with *A. euteiches*, found that ammonium carbonate (NH_4HCO_3), ammonium nitrate (NH_4NO_3), ammonium hydroxide (NH_4OH), ammonium chloride (NH_4Cl), and urea were more effective in reducing pea root rot than sodium nitrate ($NaNO_3$), calcium hydroxide ($Ca(OH)_2$), sodium carbonate (Na_2CO_3), and calcium cyanamide (fig. 29). No statistically significant differences were observed among four ammonium nitrogen sources tested. Haenseler (*131*) also stated that ammonium nitrogen retarded the development of pea root rot. Urea and ammonium nitrogen were more effective against *A. cochlioides* of sugarbeets than nitrate nitrogen (*183*).

Differences in inocula and soils used and numerous other environmental factors may account for the discrepancies with the two nitrogen forms. More studies with various kinds of soil under controlled environmental conditions are needed to determine the importance of the nitrogen form on root rot and damping-off caused by *Aphanomyces* spp.

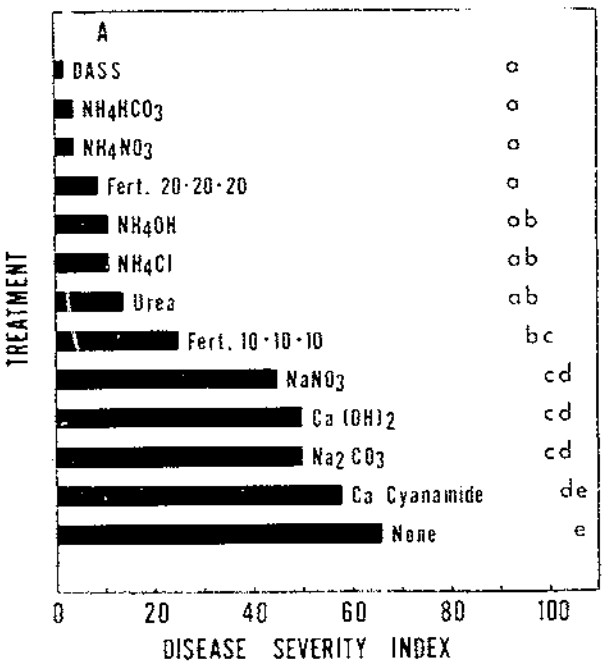


FIGURE 29.—Root rot severity in 'Early Alaska' peas grown in soil infested with *Aphanomyces euteiches* as affected by nonvolatile fungicides, fertilizers, nitrogen sources, and lime. (DASS=*p*-dimethylaminobenzenediazo sodium sulfonate.) Means with same letter are not significantly different at 5-percent level. (From Papavizas and Lewis (*244*).)

Effect of Amino Acids

Work at Beltsville (*85, 238, 239*) and elsewhere (*139, 341*) indicated that the oxidation state of sulfur profoundly affected the growth and sexual reproduction of *A. euteiches*. Despite this, the oxidation state of sulfur did not significantly affect *Aphanomyces* root rot development in sulfate-free or complete nutrient solution (*241*). These studies on the effect of various sulfur sources on the development of *Aphanomyces* root rot of peas grown in nutrient solution led to the discovery that DL- and L-methionine, and to some extent D-methionine, prevented disease development, even though they were in no way detrimental to the pathogen (fig. 30, *A*).

A continuous exposure to methionine for 25 days after inoculation was needed for complete control. Delay in adding methionine to the nutrient solution for a few days after inoculation resulted in little control. Preconditioning pea plants in complete nutrient

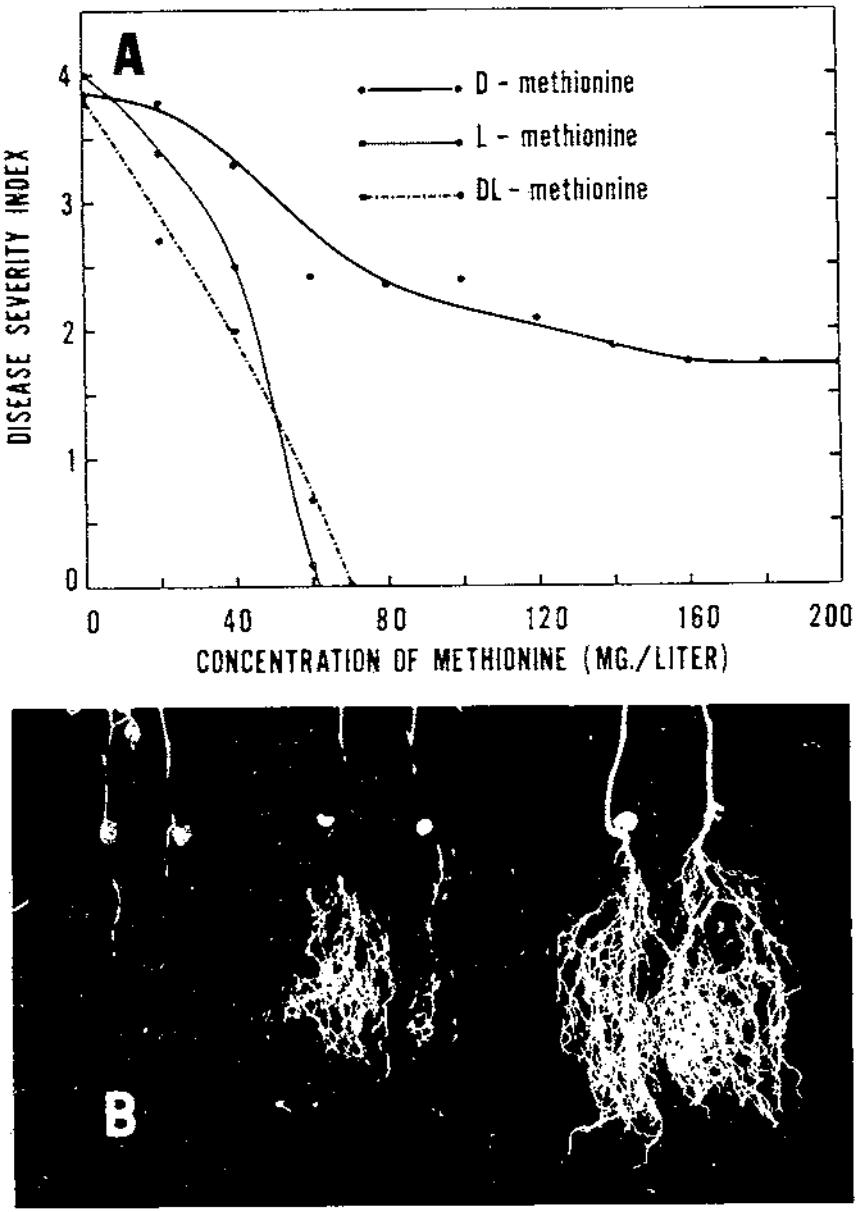


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FIGURE 30.—Severity of root rot of peas incited by *Aphanomyces euteiches* as affected by amino acids added to nutrient solution infested with zoospores of the pathogen: *A*, Effect of various concentrations of the two isomers of methionine and their racemic mixture. *B*, Effect of DL-cystine dihydrochloride and DL-norleucine: *Left to right*, nutrient solution+DL-cystine dihydrochloride, nutrient solution alone, nutrient solution+DL-norleucine. (From Papavizas and Davey (*241, 242*).)

solution supplied with DL-methionine reduced pathogenesis to some extent even when methionine was excluded from the post-inoculation solution. Ethionine, isopropionine, methylmethionine, and methyl-L-cysteine also prevented pathogenesis, whereas cysteine and cystine actually enhanced it. Several other compounds structurally related to methionine were partially or completely effective against *A. euteiches* (fig. 30, *B*). The effectiveness of L-methionine against the expression of disease symptoms on sugarbeets inoculated with *A. cochlioides* was also noted by Winner (*325*).

Further studies were made (*242*) on the effect of methyl-containing amino compounds and related substances with and without sulfur in their molecules on the expression of disease symptoms. These studies showed (1) that the oxidation state of sulfur of the compounds and root rot severity were not related, (2) that all compounds partially or completely effective against the expression of disease symptoms possessed methyl and amino groups in their molecules, (3) that the position of the methyl group in the molecule was very critical with respect to control, and (4) that the effect of some of these methyl-containing amino compounds such as methionine was not on *A. euteiches* but rather on the expression of disease symptoms.

Detailed studies on the effective compound β-methylaspartic acid (*197*), however, revealed that this methyl-containing amino acid affected zoospore germination and inhibited growth of *A. euteiches* in the absence of glutamic acid. Inhibition of *A. euteiches* growth and of root rot development was almost completely reversed by glutamic and aspartic acids and partly reversed by several other amino acids.

These studies (*241, 242, 325*) suggested a host response to the methyl group in conjunction with the amino group. They also suggested that transmethylation in plants may be somehow related to disease resistance. The concept of transmethylation was further supported by the demonstration (*86*) that pea plants were able to convert homocysteine, a precursor of methionine lacking a methyl group, to methionine, which possesses a methyl group and which, in turn, suppressed the disease.

CONTROL

Disease Avoidance

Reference has already been made to field indexing as a method of sampling soils to determine the inoculum potential of *A. euteiches* and *A. cochlioides* prior to growing a susceptible crop in

the field (see p. 86). A practical exploitation of this method was devised by Sherwood and Hagedorn (*285*) and by Reiling et al. (*253*). Hazardous fields are identified and distinguished from non-infested or slightly infested fields by determining the infectivity of field samples under controlled greenhouse conditions. This method of land selection, if performed routinely and accurately, can be valuable in avoiding severe crop losses from root rots. According to Sherwood and Hagedorn (*285*), this is the only economical, dependable "control" practice for *A. euteiches* on peas at present.

Cropping Sequences

Aphanomyces euteiches

It was early recognized that repeated cropping of a field to peas resulted in building up the population of *A. euteiches* in soil. Jones and Linford (*161*) stated that the direct relationship of the number of pea crops to root rot severity is one of the most widely recognized characteristics of the disease. In their pea disease survey, the percentage of fields with root rot increased almost linearly from 8 percent with the first crop of peas to 100 percent with the fifth crop. Thus peas could be grown for 4 successive years without root rot becoming a serious problem. If peas were planted for a fifth year, there was a 50 percent chance that the crop would be severely damaged.

In New Jersey, 100 percent of the pea plants became infected with *A. euteiches* when peas followed peas in root rot infested fields (*205, 224*). However, 26 percent of the plants were infected when peas followed alfalfa, 14 percent when peas followed oats, and 4 percent when peas followed 1 year of fallow. In New York, common root rot of peas was not eliminated by a 3-, 4-, or 5-year rotation, although the disease was reduced (*254, 255*).

Although most of the early investigators agreed that severe root rot outbreaks have occurred when peas followed peas for many years, and that crop rotation may help keep pea root rot in check (*99, 160, 161, 186, 283, 291*), the rotation of crops in controlling pea root rot has been of limited value only. The pathogen has a wide host range (*114, 185, 287*) and can also persist in soil as oospores for many years (*44, 346, 348*). Thus crop rotations with any intervals between crops of peas up to 6 to 10 years may not be expected to eradicate, not even to reduce appreciably, the root rot fungus from the soil, even though experience has shown that rotations of 6 years or more may reduce the disease to some extent (*186, 313, 333*).

The value of rotation of crops as a control measure for *A. euteiches* was questioned by Olofsson (*232*), who noted that once a heavy infestation with *A. euteiches* was established in a field in Sweden, the culture of peas became an unprofitable enterprise. He postulated that it may take 10 to 15 years to reduce inoculum density of *A. euteiches* in soil to an acceptable and safe level.

Temp (*349*) and Temp and Hagedorn (*304*) made the first systematic study of the effect of cropping practices on the *Aphanomyces* root rot of peas. Temp (*349*) found a greater decrease in the root rot index in fields planted with more than one-half their cropping sequence in corn, grain, or vegetables than in fields cropped primarily with forages. Temp and Hagedorn (*304*) indicated that crop rotations of even 10 years may not always be effective in eradicating root rot. However, these long rotations may reduce inoculum density to such an extent that a profitable pea crop can be raised.

Aphanomyces cochlioides

Crops preceding sugarbeets in a crop sequence may have a pronounced effect on the incidence and severity of blackroot incited by *A. cochlioides* (*347*). Blackroot was much less severe when sugarbeets followed corn, soybeans, and small grains (*18, 22, 70–72, 89, 146*) than when this crop followed weeds such as pigweed (*Amaranthus retroflexus*) or legumes (*70, 72*). In Montana the best treatments for seedling disease control were those in which sugarbeets were included in multiple crop sequence such as beets-barley-alfalfa (3 years) or oats-beans-alfalfa (2 years)-corn-beets (*18, 22*). The greatest amount of seedling disease occurred in unmanured 2-year rotations and in the unmanured 4- and 6-year rotations such as beets-oats-alfalfa (2 years) or beets-alfalfa (3 years)-potatoes-oats. Somewhat different results were obtained later by Morris and Afanasiev (*221*), who noted that seedling disease was lowest in sugarbeets following fallow and potatoes, highest in sugarbeets following sugarbeets, and intermediate in sugarbeets following alfalfa, beans, corn, or oats.

Not all investigators agree that legumes preceding sugarbeets increase the amount of blackroot. Buchholtz (*47*) reported that rotations of 3 to 4 years (6 years with heavily infested soils) between sugarbeet crops, with 3 to 4 years of alfalfa, were sufficient to avoid severe losses from "tip rot" presumably caused by *A. cochlioides*. Later Buchholtz (*48*) reversed himself stating that "soil infestation with *A. cochlioides* was initiated by crops of sugarbeets, but very much favored by an abundance of alfalfa, a non-host crop in the rotation."

The discrepancies in the literature on the effect of forage crops preceding sugarbeets on the incidence and severity of blackroot may be due to the fact that blackroot, seedling disease, or "tip rot" may have been caused by fungi other than *A. cochlioides* or, more likely, by combinations of fungi including *A. cochlioides*. Reactions of single pathogens or combinations of pathogens may have been different from those of *A. cochlioides* alone.

Also, Coons et al. (*72*) noted that the period when the legume sod was plowed might have been an important indirect factor determining sugarbeet stands. Experiments in various locations in the United States showed a definite relationship between the plowing date of the legume sod and the amount of blackroot in the subsequent sugarbeet crop. Considerably more blackroot was observed when the legume sod was plowed very late in the fall or in early spring than when the sods were turned under in August or September. Activity of the pathogens causing blackroot may coincide with the sugarbeet planting date when the legume sods are plowed under late in the fall or in the spring. Similar observations were also made by Afanasiev and Morris (*18*) and by Morris and Afanasiev (*221*).

Some of the results obtained by Bissonette (*334*) in the Red River Valley of Minnesota are slightly incompatible with those of other investigators. He observed less seedling disease in plots continuously cropped to sugarbeets than in plots cropped to sugarbeets for the first time. In his studies the seedling disease persisted for 3 years in all the various plots at about the same level regardless of whether sugarbeets had been cropped or not. The crop sequences usually followed in the Red River Valley (sugarbeets-barley-sweetclover, and sweetclover-summer fallow-sugarbeets) did not materially affect the populations of the seedling disease organisms.

Limited attempts were made to associate the effect by various cropping sequences with certain groups of associated soil microorganisms. In early investigations, Coons (*66*) and Coons and Kotila (*69*) noted that corn, soybeans, and small grains had a repressive effect on the blackroot-causing fungi including *A. cochlioides*. Roots and residues of legumes and pigweed favored growth of the blackroot organisms. Coons (*68*) later reported that several types of clover and alfalfa favored fungi that incited blackroot. Corn, soybeans, and small grains decreased them. Coons suggested competition to account for the control. Afanasiev (*8*) was unable to observe any significant differences in the main groups of soil micro-organisms normally occurring in soil after various crops. However, no quantitative studies were performed by Afanasiev.

Analysis of this problem was carried further by Deems and Young (*89*), who noted that after 2 years of cropping to sugarbeets, the concentration of blackroot fungi was increased to a point that 100 percent of the seedlings were infected. The prevalence of *A. cochlioides* could be associated with changes in soil mycoflora under different crops. Thus the low numbers of *A. cochlioides* in soil cropped to corn before sugarbeets were associated with high numbers of *Penicillium* spp., *Aspergillus fumigatus*, and *Trichoderma viride*. Oat soil, also low in blackroot incidence, differed from alfalfa and sugarbeet soils primarily in the predominance of *T. viride* and *A. fumigatus*. Deems and Young (*89*) postulated that corn and oats decreased *A. cochlioides* in soil by encouraging the multiplication of micro-organisms antagonistic to the pathogen.

Cultural Practices

Very little information is available on cultural practices to reduce losses from *A. euteiches* or *A. cochlioides*. Early workers emphasized the importance of early planting with early cultivars on soils of high fertility levels for reducing losses caused by *A. euteiches* (*99*, *126*). More recently Reiling et al. (*253*) suggested planting peas early to permit growth of seedlings before environmental conditions become favorable for *A. euteiches*. Drainage, phosphate fertilization, and soil loosening to promote aeration were also suggested to reduce seedling diseases of sugarbeets (*77*).

Jones and Linford (*161*) listed nine recommendations for *Aphanomyces* root rot control of peas, including land selections, cultural practices, and use of resistant cultivars. They also suggested avoidance of poorly drained soils for peas and avoidance of using "uncured" silage from pea vines, which should never be fed to animals or returned to fields as manure. No research has been done to verify these recommendations.

Organic Amendments

Relatively little information has been published prior to 1960 on the effects of plant materials in soil (organic amendments) on *A. euteiches* and *A. cochlioides*. McKeen (*200*) found that incorporation of soybean tissue into *A. cochlioides*-infested fields in Canada resulted in disease reduction. Johnson (*342*) showed that a green manure precrop of rye reduced pea root rot somewhat, whereas barnyard manure was ineffective. Although Deems and Young (*89*) dealt primarily with the effect of crop sequences, their

results left no doubt that it was the quality and quantity of each particular crop residue associated with each crop sequence that was important in the development of blackroot.

Lyda (*244*) reported that several organic amendments added to sterilized soil inoculated with *A. cochlioides* did not reduce the disease, whereas most of the same amendments added to inoculated unsterilized soil gave some, though variable, control. Addition of nitrogen by Lyda to adjust all amendments to the same level of nitrogen did not improve amendment effectiveness to reduce blackroot. He concluded that the effect of the amendments was indirect and was regulated by their effect on the entire soil microflora. Davey and Papavizas (*84*) noted that mature oat straw and corn stover, with or without supplemental nitrogen, slightly reduced root rot of peas under certain conditions.

Interest in organic amendments has been renewed by the finding at Beltsville (*235*, *236*) that cruciferous amendments, such as stems and leaves of cabbage, kale, mustard, and Brussels sprouts, when added to soil reduced *Aphanomyces* root rot of peas considerably (fig. 31). Water extracts of decomposing cabbage leaves and stems in soil did not suppress, reduce, or prevent mycelial growth, sexual reproduction, zoospore production and release, zoospore germination, or infectivity of germinating zoospores of *A. euteiches*.

In further tests by Papavizas and Lewis (*244*) and by Lewis and Papavizas (*183*), several cruciferous amendments and soybean tissue added to soil 3 weeks before planting were very effective in the greenhouse against root rot of peas and blackroot of sugarbeets. For 2 consecutive years in the field, kale reduced pea root rot by 50 percent and cabbage tissue significantly reduced root rot during the second trial year by 40 percent. Discouraging results with cruciferous amendments in the field were reported in Wisconsin (*216*), but the amount of cabbage tissue added to soil in those tests (550 lb. per acre) was less than 0.026 percent based on a 6-inch depth.

One mechanism of pea root rot suppression by cruciferous amendments may be the adverse effect on *A. euteiches* by volatile toxic substances evolved during decomposition of amendments in soil. Lewis and Papavizas (*181*) obtained direct evidence that crucifers decomposed in soil with the formation of the volatile sulfur-containing compounds methanethiol, dimethyl sulfide, and dimethyl disulfide. None of these volatile substances was evolved from decomposing corn tissue, an amendment that did not suppress root rot in soil. In addition to sulfides, isothiocyanates have been detected in vapors, distillates, and extracts of fresh or cooked

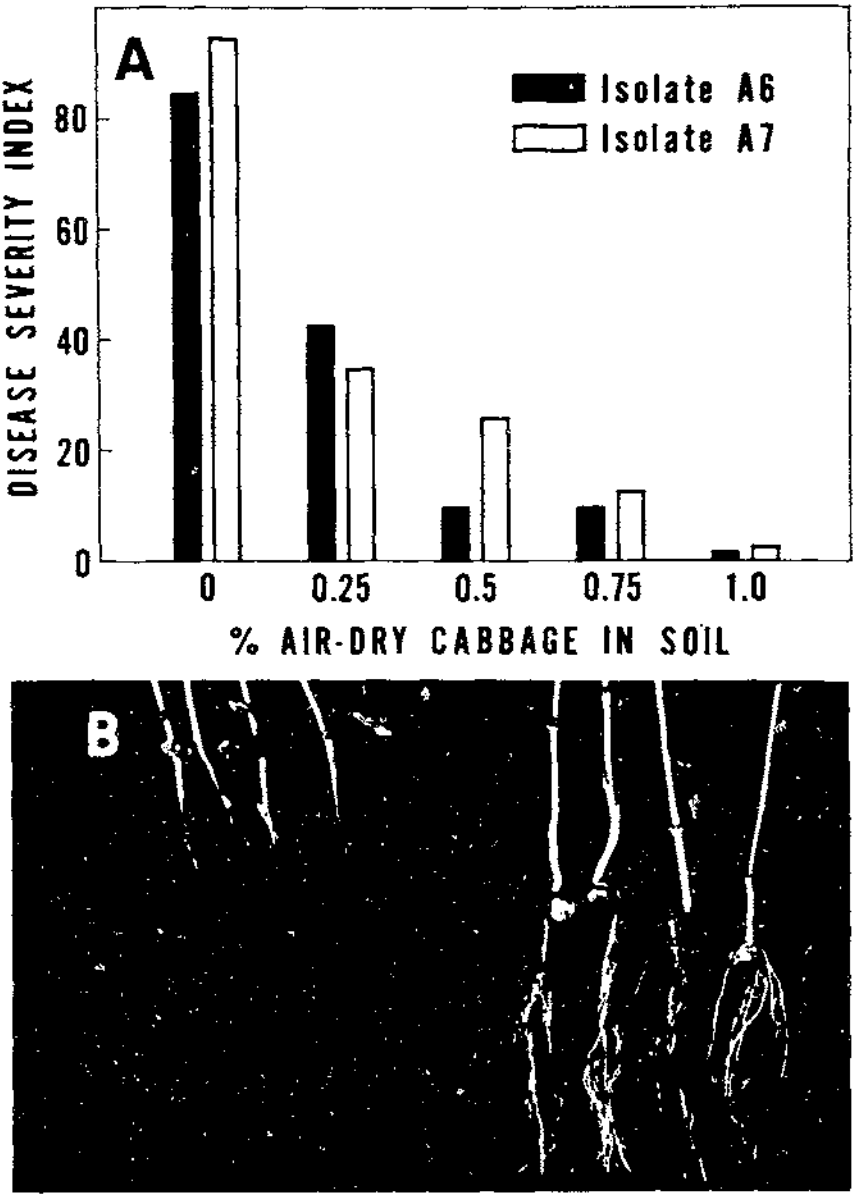

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FIGURE 31.—Root rot severity in 'Early Alaska' peas grown in soil infested with *Aphanomyces euteiches: A*, As affected by various concentrations of cabbage amendment added to soil 4 weeks before planting; *B*, as affected by cabbage amendment added to soil 3 weeks before planting: *Left*, no amendment (control); *right*, 0.5-percent cabbage stems and leaves. (From Papavizas (*235*).)

cabbage (*31, 62*). Sulfides and isothiocyanates, especially dimethyl disulfide and methyl isothiocyanate, were extremely toxic to *A. euteiches* even at concentrations as low as 0.04 p.p.m. (*182*).

Vapors from the decomposition of cabbage tissue adversely affected the morphology of *A. euteiches*, development of oospores, and mycelial growth. Vapors arising from the decomposition of corn tissue had no effect on the fungus. Lewis and Papavizas (*182*) and Papavizas and Lewis (*243*) suggested that the sulfur-containing volatiles may be implicated in the mechanism of control of *Aphanomyces* root rot of peas and blackroot of sugarbeets by cruciferous amendments.

Experimental control of the *Aphanomyces* root rot of peas in natural soil was obtained (*234, 240*) with drenches and side-dressings of DL-methionine, DL-norleucine, DL-β-aminobutyric acid (ABA), and DL-β-methylaspartic acid (MAA) applied at 70 and 100 p.p.m. within a few days before soil infestation with zoospores (fig. 32).

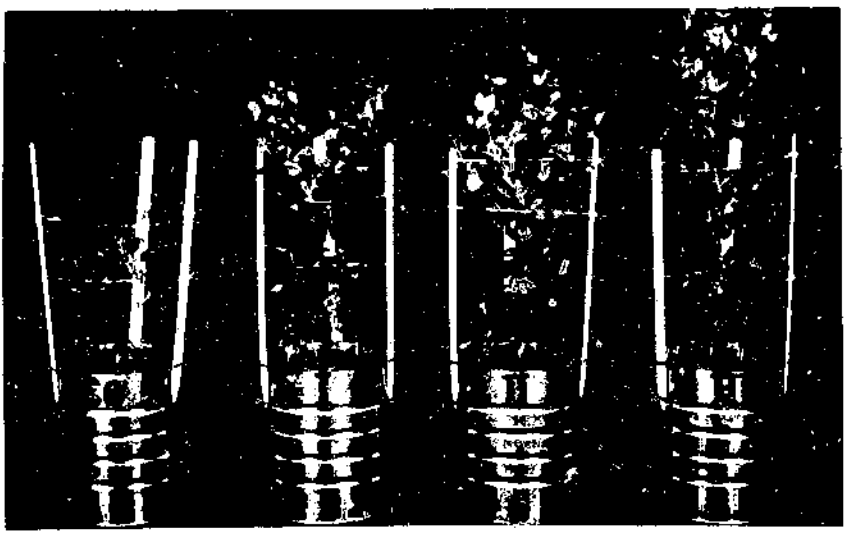

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FIGURE 32.—'Early Alaska' peas as affected by DL-β-methylaspartic acid (MAA) at 100 p.p.m. applied as a dry powder during or after planting but before soil infestation with zoospores of *Aphanomyces euteiches:* CO=none (infested control); I=MAA applied at same level as the seed; II=MAA applied at planting day to soil surface; III=MAA applied as side-dressing 3 days before soil infestation. (From Papavizas (*234*).)

ABA and MAA were more effective in reducing the disease in naturally infested soil than methionine and norleucine. The disease was not reduced if more than 3 days elapsed from soil infestation to the first drench application. The effectiveness of the amino acids was increased by combining them with potassium chloride, ammonium nitrate, or a fertilizer.

In a comparative greenhouse study of several treatments suggested for the control of *Aphanomyces* root rot of peas, Papavizas (*236*) showed that the best control, combined with plant appearance, could be obtained with ABA or MAA applied to the soil surface as a side-dressing 1 week after planting. Disease suppression by the two amino acids was equal to that obtained with the fungicide sodium *p*-dimethylaminobenzenediazo sodium sulfonate (DASS, Dexon).

Although the amino acids at relatively low concentrations were effective against pea root rot, the high cost of these materials would not permit their use in the field. In addition, no data are yet available on the performance of these amino acids in the field. Additional studies are needed to understand the behavior of *A. euteiches* in pea rhizosphere in the presence of effective amino acids.

Inorganic Amendments and Host Nutrition

Heavy applications of complete mineral fertilizers have been shown to alleviate *Aphanomyces* root rot of peas and reduce losses from the malady (*126, 127, 129, 130, 132, 224, 225, 230, 312*). In New Jersey, peas grown in field plots treated with a complete fertilizer at 2,000 pounds per acre were 73 percent taller and had 112-percent increased yield as compared with nonfertilized plots (*131*). Increase in yield and to some extent a decrease in root rot severity were almost directly proportional to the amount of fertilizer used even at concentrations as high as 2,400 pounds per acre (*224, 229*). All the principal fertilizer salts tended to retard the percentage of infection. Sodium nitrate, ammonium sulfate, and potassium chloride, however, were more effective than superphosphate (*228*). In a later report from New Jersey, 1,000 pounds per acre of the fertilizer 5-8-7, applied at weekly intervals beginning 1 day after emergence, gave the best disease control and the highest yield increases (*230*).

The beneficial effects of heavy applications of mineral fertilizers in retarding pea root rot were also noted by others (*114, 280, 282, 289, 311, 314, 342*). Most of these investigators recognized that mineral nitrogen fertilizers were the most effective in reducing

root rot but also the most injurious to pea seed (*129, 224, 227–229, 282*). Simple inorganic nitrogen salts were more injurious than complex mineral fertilizers, and the amount of fertilizer injury was roughly proportional to their nitrogen content (*129*). Even complex mineral fertilizers, however, were injurious when improperly applied or applied in large quantities. Superphosphates were practically harmless to pea seed, whereas potassium nitrate was moderately injurious (*129*).

Various ways were suggested to decrease the injurious effects of fertilizers on pea seed. The entire amount of fertilizer can be applied on top of the row just prior to seedling emergence (*129*), or less than half of the fertilizer can be applied in the row and the remainder as a side-dressing after seedling emergence (*226*). Another way is to apply a nitrogen-free fertilizer in the row and nitrogen as a side-dressing after emergence. Walker and Musbach (*314*) found that peas were better protected when the fertilizers were applied in the drill row at the time of planting than when applied in a furrow on one side of the seed, slightly removed from it.

The fact that increases in pea yield were almost directly proportional to the amount of fertilizers used led workers in New Jersey in the 1930's to speculate that fertilizers directly affected the disease and the plant rather than the plant alone (*229*). Yet in most of these reports yields were only measured without much consideration of disease severity indexes in the field. Experiments at Beltsville on the effects of fertilizers on root rot severity in soil artificially infested with *A. euteiches* were variable. Ammonium nitrate and the fertilizer 10-10-10 were completely ineffective against *A. euteiches* when added to soil at a rate to give an additional 50 p.p.m. of nitrogen to soil 3 weeks before planting (*234, 240*). In other experiments (*84, 244*), ammonium nitrate, sodium nitrate, and a complete fertilizer at 200 p.p.m. of additional nitrogen reduced pea root rot considerably. Differences in nitrogen concentrations and inoculum density of *A. euteiches* could account for the differences.

Despite all this work on mineral fertilizers and the possibility that they may reduce or retard pea root rot, fertilizers are not used today to control this disease. Even very heavy fertilizer applications may hold down the disease only during the early part of the season (*227–229, 313, 314*). Also, because of economics—beneficial results cannot offset the cost of fertilizer—peas are not normally fertilized despite demonstrations that nitrogen fertilizers increase yields and improve canning conditions (*32, 260*).

Few crops respond more profitably to proper fertilization than sugarbeets (*146*). The favorable response may be shown not only by increased yields and sugar content but also by reduction in the incidence and severity of blackroot (*16, 17, 20, 70, 72, 146, 331*). Studies in Montana (*11, 17, 18, 220*) and elsewhere (*72, 146, 171, 200, 316*) showed that proper fertilization was one of the most important practices in the control of sugarbeet seedling diseases. In some cases, only a small amount of seedling damping-off was observed in well-fertilized soils.

The field experiments on sugarbeet fertilization in relation to seedling disease control brought out some interesting differences between sugarbeets and peas. With sugarbeets, soil fertility was effective against *A. cochlioides* generally when phosphorus was increased in soil above existing levels (*20, 316*). With peas, nitrogen appeared to be the most effective of the elements against *A. euteiches*. In most of the experiments pertaining to seedling diseases of sugarbeets, an adequately balanced nitrogen-phosphorus-potassium fertilization appeared to be a prerequisite to some degree of control (*17, 19*).

The importance of phosphorus in relation to sugarbeet seedling disease control was emphasized by several other investigators (*70, 171, 322, 323*). Minimal phosphorus nutrition normally resulted in increased seedling mortality. Liberal amounts of phosphate fertilizers—three to four times the customary dose of 100 to 150 pounds per acre—resulted in increased healthy plants. Coons et al. (*72*) attributed the increase of the *Aphanomyces* form of blackroot in the 1940's in many humid areas of the United States to the progressive lowering of available phosphate. Phosphorus deficiency appears to lower the resistance of beets to *A. cochlioides*, but this phenomenon has not been positively demonstrated.

The effectiveness of phosphorus to reduce blackroot of sugarbeets appears to depend not only on the quantity and quality of the fertilizer but also on some other factors. At 28° and 32° C., superphosphate applications had no effect on blackroot (*316*). At 18°, 20°, and 24°, superphosphate reduced seedling disease markedly. Temperatures of 18° to 24° prevail during early spring when most of the sugarbeet acreage is planted. Also, phosphate added with organic matter gave better control than phosphate alone (*331*). When manure and fertilizer were used together, stands were almost perfect and yields were very high. Phosphate efficacy also depended on soil type (*316*). Phosphate added to light soils was more effective against *A. cochlioides* than the same amount of the fertilizer added to heavy soils.

Except for a short communication (*180*), no information is available on the effects of minor elements on *A. euteiches* or *A. cochlioides*. Lewis (*180*) showed that water-soluble salts of aluminum, calcium, copper, and zinc at element concentrations of 100 p.p.m. reduced *Aphanomyces* root rot of peas more than 80 percent without inhibiting pea emergence. Copper was one of the most effective elements and its effect lasted at least 2 weeks after it was added to the soil. Salts of molybdenum, boron, cobalt, barium, magnesium, or manganese were less effective and reduced pea emergence. Chelates of calcium, copper, or zinc at 100 p.p.m. did not appreciably reduce root rot. Lewis also showed that aluminum and copper at 5 p.p.m. and zinc at 100 p.p.m. prevented growth of *A. euteiches* in a liquid medium. Asexual reproduction and zoospore germination were also prevented by 1 p.p.m. of copper and 10 p.p.m. of aluminum or zinc. These materials have not yet been tested in the field.

Fungicide Seed Treatment

Pea Seed

The literature is almost completely devoid of information on pea seed treatment to control root rot caused by *A. euteiches*. Delwiche et al. (*90*) observed that seed treatments were of no benefit in the control of root rot. Johnson (*342*) noted that heavy rates of seed-protecting materials increased plant survival in infested soil. He did not mention whether seed treatments decreased root rot caused by *A. euteiches*.

Papavizas and Lewis (unpub. data) observed in the greenhouse that pea root rot caused by *A. euteiches* and *Fusarium solani* f. sp. *pisi* combined was reduced considerably by various combinations of fungicides used as seed treatments. Considerable protection of peas was afforded for up to 4 weeks from planting time. The pea cultivars Early Alaska, Freezonian, Laxtonian, Rocket, and Thomas Laxton responded well to these treatments. The seeds of Little Marvel, Wando, Dark Skinned Perfection, and Early Perfection were sensitive to seed treatments. The results from these seed treatment experiments have not yet been confirmed by field tests.

Sugarbeet Seed

Considerable amount of work has been done to control, or at least reduce, blackroot of sugarbeets by seed treatment. Campbell (*56*) reported that none of several fungicides used, including

ethylmercury chloride (Ceresan) and ethylmercury phosphate (New Improved Ceresan), gave any significant control of blackroot where the soil was heavily infested with *A. cochlioides*. Even in some cases where there was some apparent protection, this was not for the full duration of the growth of seedlings.

Early work in Montana (*6, 11, 12, 16–19, 220*) and elsewhere (*46*) showed that seed treatments with various fungicides, including ethylmercury chloride, ethylmercury phosphate, chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone), and copper sulfate, and with sodium nitrate and treble superphosphate had very little value in reducing losses from blackroot in sugarbeet fields. Adequate and properly balanced fertilization appeared to be much more important in control than seed treatment. The inability of ethylmercury phosphate alone or in combination with several protectant fungicides to control sugarbeet blackroot has also been reported by Byford (*55*).

In contrast, some researchers stated that seed treatments may be beneficial even in the field. Coons et al. (*72*) reported that nearly all fungicides tested gave significantly better stands of sugarbeets than nontreated seeds. The best control was obtained with ethylmercury chloride seed treatment. Frequently, however, in their seed treatment experiments, no significant differences could be detected at harvest between treated and nontreated plots. Coons et al. emphasized the importance of seed treatment during the initial acute phase of blackroot and its inability to reduce postemergence damping-off. Amann (*26*) showed that seed treatments with mercury compounds were effective against early infection of sugarbeets by *A. cochlioides* and other pathogens, especially when single seed of inferior quality was sown in soils that tended to crust. Gram (*118–121*) also emphasized the importance of seed treatment to reduce losses from blackroot in Denmark.

The beneficial results of seed treatment were also observed by others in the United States and Canada. Experiments by Hildebrand and Koch (*146*) and by Campbell (*57*) indicated that seed treatments with ethylmercury chloride provided some protection against damping-off in its preemergence phase. None of the treatments provided adequate protection against the postemergence phase of the disease. LeClerg (*179*) in Minnesota and Leach and Houston (*178*) in California reported good results with seed treatments. Gaskill and Kreutzer (*113*) noted that thiram (tetramethylthiuram disulfide), ethylmercury phosphate, ethylmercury chloride, and yellow cuprocide improved seedling survival in Colorado fields where damping-off was a serious problem.

Interest in sugarbeet seed treatments for blackroot control has been renewed in the 1960's as a result of the development of new nonmercurial seed protectants. In experiments by Afanasiev (*14*), a very high percentage of sugarbeet seedlings remained healthy when seed was treated with DASS at 2 ounces per hundredweight and planted in *A. cochlioides*-infested soil. When *Rhizoctonia solani* was used to infest the soil together with *A. cochlioides*, DASS was ineffective unless it was combined with pentachloronitrobenzene (PCNB). Since in nature *A. cochlioides* may rarely occur alone, it is not surprising that other seed treatments failed to produce control of blackroot in the past. Experiments by the U.S. Department of Agriculture in Michigan (*276–278*) also showed that DASS alone or with carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) resulted in the highest emergence counts. By the end of July, however, the only treatments still effective were soil treatments that included DASS.

Fungicide Soil Treatment

Aphanomyces enteiches

In the 1960's and early 1970's considerable research was done to control pea root rot with soil fungicides. Most of the fungicides tested either failed completely to control the disease or gave mediocre results. Although some chemical control has been achieved in the greenhouse and in limited field trials, none of the effective chemicals has been recommended for field application. Economics does not permit use of soil chemicals for control of pea root rot at present. Nevertheless research results with effective chemicals merit discussion so that the accumulated knowledge may be available for future studies.

Of more than a hundred fungicides and experimental materials tested in the greenhouse and field, only six fungicides reduced pea root rot somewhat in Michigan (*193*), and only in-furrow applications of 1-chloro-2-nitropropane (chloronitropropane, Lanstan) were effective in Washington (*134–136*). The fumigant chloronitropropane, which appeared to be effective also against *Fusarium, Pythium*, and *Rhizoctonia*, increased pea yields in the field and improved quality of the product by delaying the progress of the disease (*135, 136*). This fungicide, however, is lachrymatory and of high toxicity (acute LD_{50} (rat), 197 mg. per kilogram), with no tolerances permitted. It has no clearance for peas.

In addition to chloronitropropane, the fumigants sodium *N*-methyldithiocarbamate (SMDC, Vapam) and 3,5-dimethyltetrahydro-1,3,5,2H-thiadiazine-2-thione (DMTT, Mylone) have

also been tested (*244*). The use of SMDC and DMTT was suggested by the finding that sulfur-volatile compounds including isothiocyanates are liberated during crucifer decomposition (*31, 62, 181*). Methyl isothiocyanate (MIT) is presently used in solutions with chlorinated hydrocarbons (Vorlex) and is the active material resulting from the decomposition of SMDC and DMTT (*189, 228*).

In greenhouse tests pea root rot was reduced by more than 90 percent when soils infested with *A. euteiches* were fumigated with carbon disulfide, dimethyl disulfide, MIT, or methanethiol (*182*). Very good control of *A. euteiches* was obtained in the greenhouse and field with SMDC and DMTT at 50 to 200 p.p.m. These fumigants were effective with several pea cultivars and at a soil temperature of 17° to 32° C. In a field experiment in Wisconsin (G. C. Papavizas and J. A. Lewis, unpub. data), SMDC and DMTT increased pea yields by approximately 30 to 50 percent. Olofsson (*232*) also observed that DMTT controlled pea root rot in Sweden.

At present, it cannot be stated whether the use of such fumigants as SMDC and DMTT will become agriculturally feasible and economically profitable. In addition, these materials have not been tested adequately in the field.

Except for DASS, no nonvolatile fungicides are known to be effective against *A. euteiches* in soil (*244*). In some tests DASS failed to reduce pea root rot. In other tests DASS was consistently effective against *A. euteiches* (*215, 216, 236, 345*) without affecting the soil mycoflora (*25*). Although chemical analyses of DASS indicated that substantial residues of the fungicide remained in soil for long periods (*25*), the performance of this material was less striking in the field than in the greenhouse (*215*). The inability of DASS to act effectively in the field was attributed by Pivaral (*345*) to uneven distribution of the fungicide in soil.

Although Mitchell and Hagedorn (*215*) failed to obtain a practical disease reduction with DASS in the field, they made the important observation that disease reduction by DASS was less when peas were planted immediately after the DASS treatment than when planted at long intervals after fungicide applications. In subsequent experiments, fall applications of small amounts of DASS at 30 pounds per acre or less resulted in considerable root rot control and increased yields in the spring (*216, 217*). The lasting effectiveness of DASS was attributed to its ability to persist in soil for several months up to a year (*25*) and to reduce the propagules of *A. euteiches* during the initial application (*217*). Approximately 1 to 2 μg. of residual DASS per gram of soil were required to completely inhibit zoospore formation. This residual quantity in soil

could be provided by applying 30 pounds per acre in the fall. Despite these encouraging results, Mitchell and Hagedorn (*217*) concluded that the application of even 30 pounds per acre of DASS in the fall would not be economically feasible in pea culture.

Aphanomyces cochlioides

In the seedling disease complex of sugarbeets, *A. cochlioides* was the most difficult organism to control with chemical soil treatments (*46, 152*). Most of the results reported on the chemical control of *A. cochlioides* appeared initially encouraging because the research dealt mainly with situations where *A. cochlioides* was either absent or represented by low inoculum density. Thus chemical soil-row treatments in the greenhouse (*152*) or field (*117, 198*) with various fungicides gave good control of seedling diseases caused by three to four pathogens. The fungicides were either ineffective against *A. cochlioides* or this pathogen was not mentioned as being involved in the disease complex. The European literature also contains reports with encouraging results on chemical control of seedling diseases without special mention of *A. cochlioides* (*208*).

Thiram was among the earlier materials tested in soil-row treatments to control seedling diseases of sugarbeets (*117, 147–150*). Thiram at 3 to 4 pounds per acre, mixed with commercial fertilizers of low nitrogen content and placed as close as possible in the zone through which seedlings emerge, proved the most effective of several chemicals tested in reducing preemergence and postemergence damping-off (*147, 149*). The degree of control was closely associated with the extent to which this fungicide could be mixed with the infested soil around and above the seed. Soil treatments with thiram mixed with fertilizers, however, have not become adopted in commercial practice despite the fact that thiram was effective in relatively small amounts and despite its ability to remain active in soil for long periods.

In addition to thiram, DASS showed promise in the control of seedling diseases of sugarbeets in the United States (*14, 177*), Canada (*79*), and elsewhere (*323*). DASS has been used experimentally alone, with phosphate fertilizers where the disease was increased by soil phosphate deficiencies (*323*), or with PCNB when *Rhizoctonia solani* was also involved in the disease complex (*14*).

Results under controlled conditions showed that DASS could inhibit asexual sporulation of *A. cochlioides* at 2.5 p.p.m. when sugarbeet seedlings infested with the pathogen were placed in water cultures (*151*). Occasionally, however, field or greenhouse

results with DASS were somewhat variable. The effectiveness of DASS in the greenhouse against *A. cochlioides* was shown to be dependent on the type formulation and the mode of its application (*183*). In more recent comparative field studies with several new fungicides (*276–278*), the greatest degree of control of *A. cochlioides* was obtained with DASS and chlorothalonil sprayed over rows in small amounts. By the end of July DASS was the only treatment that still appeared to be effective. DASS, however, has not been recommended for commercial use and it may not be economically profitable. Its activity is also quickly destroyed by exposure to sunlight (*151*).

Disease Resistance

Peas

In the 1920's and early 1930's some pea cultivars were shown to be more tolerant than others to *A. euteiches* (*125, 159–161, 186, 206, 207*). No cultivars were reported to be immune or highly resistant to the disease. No attempts were made at that time to search for new sources of resistance or to use any tolerant lines or cultivars in any systematic breeding program. In the early 1950's Johnson (*342*) compared the susceptibility to *A. euteiches* of 22 pea introductions with standard cultivars. He found that 12 of the introductions had greater tolerance to root rot than the commercial cultivars tested. Tolerance of the plant introductions was expressed as a greater ability to survive and grow in the presence of the pathogen than the ability of the commercial cultivars.

In the middle 1950's rigorous screening and testing programs began in the agricultural experiment stations in Michigan, Minnesota, New York, and Wisconsin to develop pea cultivars resistant to *A. euteiches* and to other root-rotting fungi (*61, 168, 190, 191, 195, 281, 335, 337*). In Michigan, Lockwood (*190, 191*) and Lockwood and Ballard (*195*) noted that all commercial pea cultivars were susceptible to *A. euteiches*. Of approximately 800 pea introductions tested in their standardized greenhouse experiments, several were tolerant or "incompletely" resistant to the pathogen. No immunity or high degree of resistance was observed among the introductions tested. In New York, of the entire collection of pea introductions screened for *Aphanomyces* root rot resistance (*5*), only four introductions showed good tolerance to *A. euteiches*. Publications from Minnesota refer to at least two selections as being tolerant to *A. euteiches* (*61, 168, 335*).

Laboratory techniques were developed in Minnesota for evaluating resistance of pea cultivars and breeding lines to *A. euteiches*

(*61, 168*). The technique is based on the development of larger numbers of oospores by *A. euteiches* in excised, zoospore-inoculated root tips of susceptible lines than in root tips of resistant ones. Morrison et al. (*222*) refined the excised root tip technique and evaluated resistance in several breeding lines and pea cultivars to *A. euteiches* by the number of oospores formed in infected excised root tips. Lower numbers of oospores of *A. euteiches* were formed in root tips of resistant genotypes than in susceptible ones. The refined technique may expedite testing of great numbers of lines and cultivars of *Pisum sativum* for resistance to *A. euteiches* before resistance can be determined in the field.

Limited attempts were made to incorporate the tolerance observed in some plant introductions into commercially acceptable pea cultivars (*167, 191*). Some of the efforts yielded uniform lines of peas bearing seed of commercial types and with resistance levels comparable to those in pea introductions. Nevertheless no resistant cultivars of peas have been released from these programs. The failures of the screening efforts and breeding programs to produce commercially acceptable cultivars resistant or tolerant to *A. euteiches* may be due to (1) the low levels of resistance found in plant introductions and the inability of this type of resistance to express itself in the field, (2) the presence of races of *A. euteiches* with differential degrees of pathogenicity, and (3) the synergistic action of more than one pathogen responsible for pea root rot in the field. Development of multiple resistance in peas to withstand complex root rot situations would be much more difficult than development of resistant cultivars to *A. euteiches* only.

Sugarbeets

In 1940 and 1941 Coons (*67*) noted that inbred U.S. 216, a leaf spot-resistant strain, as well as sugarbeet hydrids and synthetic cultivars in which U.S. 216 occurred as a component, showed considerable resistance to blackroot. These observations led to the development of an intensive program of selection and breeding for resistance to *A. cochlioides* by the U.S. Department of Agriculture and by several sugar companies.

The remarkable advances made from the early 1940's to the middle 1950's were reported in several research papers (*40, 42, 48, 67, 72, 74–76, 93–97, 112, 148, 339*) and summarized in two reviews (*73, 98*). These early efforts resulted in the development and release of U.S. 400 and U.S. 401 and American Crystal No. 3 and No. 5, commercial multigerm cultivars tolerant to *A. cochlioides*. At the same time, the U.S. Department of Agriculture

selected 48B3-00, 51109-0, 51-B1-00, and Accession 1191, all with good tolerance to *A. cochlioides* (*42, 73*). The use of cultivars tolerant to *A. cochlioides* from 1944 to 1952 resulted in considerable increase in yields. Most of these selections were abandoned in later years either because they were no longer tolerant or because better selections were made.

A considerable amount of research was performed during the 1950's and 1960's in the greenhouse and field to obtain tolerant or resistant cultivars to *A. cochlioides* (*13, 21, 23, 40, 264*). Some studies were disappointing, but others led to the development of new lines that were used in future work.

In 1957 the U.S. Department of Agriculture undertook a program for testing breeders' strains of sugarbeets. Thousands of strains were tested at Beltsville and elsewhere. Most of them were derived from plants selected for resistance to *A. cochlioides* in field trials. Multigerm, monogerm, and monogerm-multigerm hybrid types were included along with commercial types. Usually the semiresistant U.S. 400 or U.S. 401 was included as a comparison. The materials were exposed to *A. cochlioides* in the greenhouse (*63, 64, 269, 272*).

These studies resulted in an extraordinary amount of information, which is summarized as follows:

(1) Improvement in blackroot resistance can be achieved in monogerm progenies developed through backcrossing and selection.

(2) Most of the multigerm diploid, multigerm tetraploid, monogerm diploid, and monogerm-multigerm hybrids were more resistant than U.S. 400 and U.S. 401. The monogerm-multigerm hybrids SP-59485-1 and SP-59495-1 were outstanding in their performance.

(3) About 50 percent of the selected plants produced progenies more resistant to blackroot than the parental lines.

(4) The percentage of selected plants that gave progenies with higher resistance to *A. cochlioides* was less from F_1 hybrid lines than from open-pollinated lines.

Two selections, SP-62490–1 and SP-62501–1, were more resistant to *A. cochlioides* than the parental stock (SP-603555–1) (fig. 33). Some of the lines also differed in degree of susceptibility to the chronic phase of *A. cochlioides* (*268*).

These results disagreed with those of Schneider and Gaskill (*274*), who noted that most of their entries were less resistant than the check cultivar U.S. 401. About 93 percent of the materials tested, which were mostly foreign introductions, were more susceptible to *A. cochlioides* than U.S. 401. These researchers also observed that annual types of *Beta vulgaris* were less susceptible

PN-3598

FIGURE 33.—Sugarbeet lines showing different degrees of tolerance to *Aphanomyces cochlioides: Left to right;* U.S. 401, SP-603555-1, SP-62490-1, and SP-62501-1; last two are progenies of plants selected from SP-603555-1. (From Coe and Schneider (*64*); courtesy of G. E. Coe.)

to blackroot than biennial types and that susceptibility to *A. cochlioides* tended to be associated with susceptibility to *Rhizoctonia solani*. Coulombe (*80*) also noted that table beet cultivars were less susceptible than sugarbeet cultivars to both *A. cochlioides* and *R. solani*. The results of Coe and Schneider (*64*), on the other hand, contradicted the earlier conclusions (*41*) that tolerance to *A. cochlioides* is conditioned by a single dominant Mendelian gene. In their experiments many genetic factors appeared to be responsible for tolerance.

From a practical standpoint all the selection and breeding efforts produced new improved lines of sugarbeets with substantial resistance to *A. cochlioides*. The multigerm hybrids U.S. H2, U.S. H3, U.S. H4, U.S. H5, and U.S. H6, developed at Salinas, Calif., gave excellent performance in the early 1960's. U.S. H2 increased yields 22 percent in California and U.S. H6 10 percent over that of U.S. 75, an open-pollinated cultivar (Sugarbeet Investigations, unpub. data).

Also, from the practical standpoint, the development of monogerm hybrids suitable for most regions of sugarbeet production is considered a great accomplishment in the sugar industry and

seed-producing enterprises since 1963. From a small production of an experimental hybrid in 1955, the percentage of monogerm seed steadily increased. In 1962 the sugarbeet crop in the United States was almost 86 percent monogerm. In the 1965 crop, monogerm lines comprised 99.3 percent of the total seed used. Resistance to *A. cochlioides* was incorporated into several monogerm lines, such as U.S. H7 and U.S. H8, released for seed production in 1964, and U.S. H20, released for the Great Lakes region in 1967. The monogerm hybrids excelled not only in resistance to *A. cochlioides* but also in yields and quality.

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