

CHAPTER 27. Parasites and Pathogens: Root Rot of Peas

The number of Saprolegniaceae found guilty of trespass in other living organisms is not great, and the few culprits that are known to do so are facultative parasites. Nevertheless a broad degree of parasitism is here, from those individuals invading presumably healthy tissue as primary pathogens, to supposedly self-parasitic ones as exemplified by *Saprolegnia megasperma* (Nolan, 1975a).

It is not to be thought *infra dignitatem* to give attention to the pathology of the saprolegniaceous fungi, since for some, this is their way of life. Accordingly, in this and the remaining chapters we will consider such features of their existence as infectivity, epidemiology, and -- but briefly -- control. The physiology of the growth and reproduction of the parasites themselves, and their ultrastructure as well as other aspects are treated in prior chapters; whatever else these creatures may be they are respectable water molds.

Most (if not all) parasitic water molds would qualify as primitive ones since they possess at least three of the capabilities circumscribed by Wheeler (1968:203). The majority cause necrosis of the host cells, they apparently can grow saprotrophically under natural conditions, and they have broad host spectra. In spite of their alleged primitiveness one would expect them to succeed eminently, being self-propelled by nature. With perhaps the single exception of those species committing misdemeanors among fish, young and old alike, none has attained major status among the known pathogens. Nonetheless, these parasites inflict injury, and it is important in the interests of completeness to explore this aspect of the family's members.

Though not directly dealing with the Saprolegniaceae, two papers considering very special conditions attendant on the establishment of disease are important background sources. Bracker and Littlefield (1973) treat commendably the subject of interfaces between the host and pathogen. Held (1973) deals with the establishment of a parasitic relationship: attraction of the infective unit to the host, attachment of that unit to the host surface, entry (perhaps the most critical point -- at arrival -- in the mode of a parasite's existence), and endogenous development.

APHANOMYCES EUTEICHES, AND THE ROOT ROT OF PEAS

Although it was not until 1925 that Jones and Drechsler described *Aphanomyces euteiches*, and cited it as the agent of root rot of peas (*Pisum sativum* L.), doubtless the disease had been recognized but its cause not known (Haenseler, 1923) prior to this time. The common name "root rot" was firmly established in 1936, when Wakefield and Moore specifically applied this term to the disease caused by *A. euteiches*. They reserved the name "black root rot" for the pathological condition caused by *Thielaviopsis basicola* (Berk. and Br.) Ferraris. Root rot occurs to some extent wherever peas are grown (although it is not always responsible for the disease in some years, according to J. L. Lockwood *et al.*, 1957), and the host plant is susceptible at any stage in its development. Lipman (1925), among others, believed that the earlier in the host's

development that it became infected the more severe would be the disease. The early symptoms, at least, are not particularly prominent, nor always readily recognized. Haenseler (1928) for instance, calculated that infection by *A. euteiches* had a greater effect on the dry weight of the invaded host than on its height, hence the general appearance of the mature pea plant was not in fact a reliable indicator of its freedom from the disease.

Early indicators of infection are softened, water-soaked areas on the root and epicotyl. Under proper conditions *Aphanomyces euteiches* invades the root cortex and eventually contributes to its disintegration. In advanced stages, the cortex discolors, but this is a symptom attributed to infestation by secondary organisms. It has been reported by most workers that the stele of the pea plant is not invaded, as gross symptomatology suggests. However, Cunningham (1961), and Cunningham and Hagedorn (1962b) found that hyphae of the causal organism certainly could penetrate into the phloem. Mycelium of *A. euteiches* can invade the apical meristem region of the host thus retarding root growth.

At the point of entry into roots, the fungus usually does not cause any yellowing of tissue, but Jones and Drechsler (1925) reported that it did so if it penetrated stem tissue.

The intensity of symptoms of pea root rot evidently varies with the extant environmental conditions (Papavizas and Ayers, 1974). At ground level, symptoms of stem rot may appear, and concomitant with the spread of infection in the root system, the lower leaves may become necrotic and somewhat brittle. Wilting can be a symptom in plants that have become infected prior to the time they develop three or four stem nodes, but older plants can mature without exhibiting any external symptoms. The only certain diagnostic feature of the disease appears to be the presence of the oogonia of the *Aphanomyces* in the cortex, although the fact that this tissue in infected plants separates easily from the vascular cylinder is a useful recognition characteristic of secondary importance.

That some plants could become infected by *Aphanomyces euteiches* and yet survive to maturity, KenKnight (1944) stated, was related to the duration of the conditions favoring the progression of the disease at the time of its inception, or to the "amount" of the causal agent in the soil. Infection does not spread in the field from plant to plant, since migration of spores usually does not exceed $1/2$ inch (about 13 mm) in the soil (Haenseler, 1925; Lipman, 1925). It has been suggested (Lipman, 1925) that the spores of the causal agent are infective for the entire time they are motile in the soil, a period alleged to be 3-4 days.

The extant literature leaves no doubt that the disease known as root rot of peas can be caused by fungi other than *Aphanomyces euteiches*. Even Drechsler (1954b) was at first uncertain of the identity of an *Aphanomyces euteiches* P. F. 2 isolated by Muers (1928) from the roots of *Viola tricolor* L. and *V. cornuta*, and subsequently studied also by van Eek (1938). The latter concluded that Muers' isolate of an *Aphanomyces* was not the cause of root rot in pansies, but believed that *Brevilegnia gracilis* and *B. macrospora* were strongly parasitic to these plants. It happens, however, that van Eek's *Brevilegnias*

were species of *Pythium*. In any case, Drechsler (1954b) concluded that Muers' fungus was *A. cladogamus*. The specimen that he had isolated from pansy roots, Muers found, would not infect peas, yet he persisted in considering it to be *A. euteiches*. Buisman (1927) also isolated from decaying roots of *Violas* a specimen of *Aphanomyces* that had the characteristics of *A. euteiches*, but would not infect peas.

Species of *Fusarium* and *Pythium* also are known to invade pea roots. To be able to distinguish roots infected by *Fusarium* species from those harboring *Aphanomyces euteiches* Harter *et al.* (1934) pointed out, it was necessary to look at the vascular cylinder of the root. The stele of plants infected by *Fusarium* was a deep red; there was no discoloration of this tissue if *A. euteiches* infected the root. Drechsler (1925) believed that *Pythium* species were only secondary invaders of pea roots, the mycelium of the *Aphanomyces* having killed the cortical tissue and thus provided easy access to pythiaceae hyphae.

The various studies by Solberg attributed root rot of peas in Norway to still other organisms. In 1925 she reported that the principal fungus associated with pea root rot was a *Pythium* acting as a secondary invader, but in 1926 claimed that the cause of the disease was a bacterium -- *Bacillus radicumicola* -- with *Aphanomyces euteiches* entering the diseased plants only secondarily. In a short time, however, Solberg (1927) decided that the cause of root rot was a myxobacterium (a species of *Polyangium*) since she saw in infected pea roots polyhedral, cyst-like bodies. Louise Solberg (as L. S. Heimbeck) again revised her view of the root rot of peas in 1954, in this instance citing the cause of the brownish rot in pea roots as B type L forms of bacteria (Tulasne, 1951). Heimbeck inoculated pea plants with *A. euteiches* and although the mycelium invaded the cortex, no brown lesions developed. In a control plant, the uninoculated roots became necrotic, and Heimbeck concluded that the L forms were originally in the seed. It was her opinion that the hyphae of *A. euteiches* (among other root-invading fungi) were vectors for the L forms, and insured that these organisms gained access to the root's vascular tissue.

Several papers provide information on general symptoms of *Aphanomyces* root rot of peas, the nature of the disease, and its prevalence in various geographic regions. Prominent among these are the accounts by Drechsler (1925), Haenseler (1925, 1926; very early studies), Harter *et al.* (1934), Lipman (1925, 1926), Lloyd and Lockwood (1961), W. H. Martin (1931; one of a series of annual reports), Reinking (1942), Walker and Hare (1943), Olofsson (1967), Walker and Snyder (1933), and Zaumeyer (1962). In addition, there are numerous brief reports of the disease from the United States and abroad as well as extensive ones that treat symptomatology and prevalence, among other things. These publications are examples: Clinton (1920: unnamed, but description of the fungus suggests *Aphanomyces euteiches*; 1934), Fenwick (1969: root rot is consistently present, but not as important as other pea diseases in Idaho); Haenseler (1924), Linford (1929: known distribution in pea-growing regions of the U.S.), B. L. Richards (1925), Starr (1932), and Weimer (1940). Some of the principal accounts of pea root rot abroad are those by Fukumishi *et al.* (1976) and Yokosawa *et al.* (1974) in Japan; Jørstad (1928), Solberg (1925), and Sundheim (1971) in Norway; Labrousse (1933, 1934:

as root collar rot) in France; Muers (1928) in the Netherlands, Olofsson (1967) in Sweden, and Kotova (1969, 1971, 1976) in Russia.

FACTORS INFLUENCING DISEASE DEVELOPMENT

SOIL MOISTURE

No matter whether the influence of soil moisture on the severity of pea root rot is put in negative or positive terms, there is almost unanimous agreement among investigators that the disease is favored by a high soil water level, poor drainage, heavy (and thus wetter) soil, or wet seasons (Geach, 1936; Geard, 1961; Harter *et al.*, 1934; Jones and Linford, 1925; KenKnight, 1944; Linford, 1929; J. L. Lockwood and Ballard, 1959; R. T. Sherwood, 1958; Starr, 1932; Walker and Hare, 1943; Walker and Snyder, 1933; Weimer, 1940), or, conversely, is retarded, is least damaging or held to be unimportant in well drained soils or dry seasons (H. G. Johnson, 1953; Reinking, 1942; Reinking and Newhall, 1950). The precise soil moisture level at which pea root rot due to *Aphanomyces euteiches* reaches its maximum intensity, or the minimum soil water content that permits disease development, is somewhat in dispute. According to Haenseler (1925) no infection of peas by the fungus occurred at a soil moisture level of 20% of saturation, and 30% seemed to be the minimum (Haenseler, 1926) level at which the disease condition could develop. Observations by P. G. Smith and Walker (1941) were in agreement, but Burke *et al.* (1969b) believed that severe root rot was not possible until the water holding capacity of the soil approached saturation. In Swedish pea fields, Olofsson (1967) observed that there was no infection when the soil moisture was 45% of the water holding capacity. According to Jones and Drechsler (1925) disease severity was not coincident with an important role for soil moisture.

Scharen (1960a) explained that the oospores (the principal source of inoculum) of *Aphanomyces euteiches* germinated in anaerobic conditions, and this correlated well with reports that the disease was most prevalent and severe in persistently wet weather or poorly-drained soils. Viable specimens of *A. euteiches* have been recovered from soils held experimentally at 66, 100, and 160% field capacity (R. T. Sherwood and Hagedorn, 1962).

SOIL TYPE AND FIELD HISTORY

Although Reinking (1942) concluded that soil type was not a limiting factor in the occurrence and prevalence of pea root rot, others found that some correlation did in fact exist. Geach (1936) demonstrated experimentally that root rot caused by *Aphanomyces euteiches* was more severe in peas planted in red loam than in an alluvial soil, and Temp (1966) found that the severity of root rot declined more rapidly in test plants grown in some silt-loam soils than in those propagated in muck. More plants in clay and clay loams were infected than were those planted in fields of silt-loams or lighter soils, according to Jones and Linford (1925). However, none of 27 soil types

examined by these two investigators was free of the causal agent. In Wisconsin, Jones and Drechsler (1925) determined that root rot was more severe in red clay soils than in any other soil type; Drechsler (1925) also noted that infection was more prevalent in heavy than in light soils. It has been shown in some experimental work by Alconero and Hagedorn (1967) on clay loam, sandy loam, silt loam, and muck soils that these do not favor invasion of *Pythium*-incited roots by *Aphanomyces euteiches*. However if such soil type were mixed with sand or cornmeal, the loss of seedlings rose. Soil texture modified by krillium has been found to reduce pea root rot slightly under greenhouse conditions (H. G. Johnson, 1953).

Some experimental work by Burke *et al.* (1969a), J. L. Lockwood (1959, 1960c), Scharen (1960a, b), R. T. Sherwood (1958), and Temp and Hagedorn (1967) yielded information on various soil conditions (other than physical type) as factors in the establishment and subsequent severity of root rot.

Scharen (1960a, b) experimented with germinability of *Aphanomyces euteiches* oospores embedded in plant debris particles. These oospores were put into permeable casings and then buried next to roots of peas and other plants (nonleguminous), both in sterile and unsterile soil. There were higher percentages of germination in encased oospores placed near pea roots than in those lying adjacent to roots of other plants, and in sterile rather than nonsterile soil. He concluded that natural soil was fungistatic. Utilizing soil columns (inoculated with the *Aphanomyces*) R. T. Sherwood (1958) performed some experimental work on the recovery of the fungus from soil. He failed to reisolate *A. euteiches* from unautoclaved soil, or from autoclaved soil seeded with nonsterilized soil containing propagules of the fungus. He concluded that competitive or antagonistic organisms prevented *A. euteiches* from infesting sterile soil, and therefore it did not spread to any extent in normal field soil. Hyphae of *A. euteiches* were readily lysed when buried in untreated and nonsterile field soil, according to Lockwood (1959, 1960c).

Using a special soil layering system in pots, Burke and associates (1969a) investigated in growth chambers and in field plots the migration of roots into infested soil, and the accompanying "movement" of *Aphanomyces euteiches*. In any cases where growing pea roots pushed into infested soil, they became infected, but if they grew progressively from an infested into a noninfested soil they essentially avoided the pathogen. Those portions of the roots within infested soil might show severe cortical damage yet the parts of the same roots growing into sterile (steamed) soil were free of infection. The fungus did not move up into the plant from the root system. Where roots were delayed in their growth in infested soil (as the result of some mechanical obstruction) disease severity increased measurably.

In 1929, Linford pointed out that pea root rot was especially troublesome in soils that were repeatedly planted to peas (*see* section on control and suppression). KenKnight (1944) commented that fields apparently did not become heavily infested with the causal agent until two or more crops of peas had been sown on them, and Boosalis and Scharen (1959) found that no root fragments from soils with a low disease

index contained oospores of *Aphanomyces euteiches*. Their observation can be construed as correlating well with a field history of few pea plantings.

Since populations of *Aphanomyces euteiches* in soil are in a constant state of change, an assay method to measure the population reliably at any given time is necessary. Such a method was devised by J. E. Mitchell and colleagues (1969). The resulting data demonstrated that the number of seedlings infested was proportional to the amount of debris in the soil: the more plant debris the higher was the disease incidence.

Soils from 128 fields with known histories of specific cropping practices were assayed by Temp and Hagedorn (1967) for *Aphanomyces* root rot incidence. They rated the fields according to an arbitrary index: fields with an index of 0-49 were considered safe for planting peas; those rated 50-69 were questionably useful for pea crops, and ones with an index of 70-100 were unsafe for peas, that is, there would be a high disease incidence expectancy. Temp and Hagedorn demonstrated conclusively that there is a strong positive correlation between the number of pea crops grown on the soil, and the disease incidence. Roots of pea plants sown in soils with a high index (70-100) universally had oogonia of *A. euteiches* in them, but those of plants grown in low index soils had fewer oospores. Fields with and without a history of pea root rot were examined by Burke *et al.* (1970). The index of *A. euteiches* was generally higher in fields where root rot had been found than in areas not known to harbor the disease organism, but there were exceptions. The fungus (detected by the incidence of seedlings infected when grown in samples of the soil) was prevalent in plowed soil layers of fields with a root rot history, but either was sparse or absent in the subtending unplowed soil.

SOIL FERTILITY

Laboratory studies -- such as those by Papavizas and Davey (1963a) -- on the influence of mineral nutrition on the development and severity of *Aphanomyces* root rot of peas are treated in a subsequent section on physiology and parasitism. Fertility level in the soil also influences the disease.

The addition of nitrogenous fertilizer to the soil, Geach (1936) reported, was effective in controlling root rot of peas in Tasmanian fields, while liming the soil favors both the growth of the host and pathogen (Haenseler 1926). A high soil fertility level (heavy application of manures or inorganic fertilizers) promotes yield in spite of the presence of the disease organism. Proper fertilizing, Harter *et al.* (1934) recommended, provided vigorous plants that would escape infection; Reinking (1942), Walker and Musbach (1939), and Zaumeyer (1962) concluded likewise. Potash reduces the incidence of the disease (Geard, 1961), and it has been determined experimentally (Wade, 1955) that the growth of pea plants is much better in *Aphanomyces*-infested, waterlogged fields if the soils are rich in potassium than if they are deficient in this element.

It appears that nitrogen level also is critical to the development and incidence of root rot of peas. In field tests with commercial fertilizers, P. G. Smith and Walker (1941)

noted higher disease incidences in unfertilized plots and in those lacking nitrogen than in fully fertilized ones. Ammonium nitrogen applied to infested test plots planted to peas resulted in increased root rot, Carley (1969) reported, but nitrate nitrogen tended to reduce the disease incidence. A low nitrogen but high phosphorus and potassium level in soil seems to encourage disease prevalence (R. T. Sherwood, 1958). According to J. A. Lewis (1973b) certain mineral salts suppress the disease severity index, and fertilization evidently does not antagonize this suppressive effect.

SOIL TEMPERATURE

Little in the way of specific information on temperature effects and the severity and prevalence of *Aphanomyces* root rot of peas is at hand. Haenseler (1925) reported that low temperatures delayed the inception of the disease, and Reinking (1942) recommended early planting of pea crops, to take advantage of the retarding effect of cool weather on growth of the *Aphanomyces*. Linford and Vaughn (1925) and KenKnight (1944) related increased damage to plants and high disease incidence to warm weather (accompanied by high moisture).

A few investigators, either by direct observation or experimental work, have explored more precisely than the foregoing the relationship of temperature to pea root rot incidence. The results of the various studies are not entirely in agreement. According to Jones and Drechsler (1925), the optimum temperature for infection of susceptible pea plants by *Aphanomyces euteiches* is between 15 and 30 °C. Haenseler (1926), however, found that unless the soil temperature was at least 14 °C for several days, infection would not develop in field plantings. A field soil temperature of 60 °F (17 °C) favored root rot, Walker and Snyder (1933) reported, and the results of a series of experimental tests by P. G. Smith and Walker (1941) indicated that maximum infection took place when the soil temperature was at 24° or 28 °C, but that there was no infection at 12 °C. The work reported by J. L. Lockwood (1960a) appears to be in agreement: the lowest disease severity index of experimentally inoculated plants occurred at 16 °C, while the highest indices were reached at 24° and 28 °C. Data from R. T. Sherwood's (1958) experimental inoculations also confirm prior reports that higher temperatures favor pea root rot development (at least in greenhouse and laboratory tests).

In an attempt to determine the effect of temperature on the infection process in pea root rot, Cho and King (1963) used infested, excised root tips (inoculated by motile spores of *Aphanomyces euteiches*), and calculated the number of oogonia that developed in the tissue after a suitable incubation period. There were no oospores in inoculated root tips incubated at 50 or 40 °C, but at 20°, 25°, and 30 °C there was very rapid development of infection as expressed by the density of oogonium production *in vivo*. Although these data from the work of Cho and King came from laboratory experiments they coincide well with field observations. As Burke and co-workers (1969b) found, colonization of pea roots experimentally inoculated with *A. euteiches* was retarded at 16 °C, but the incidence of infection was about the same as that measured in experimental

plants held at 20°, 24°, or 28 °C. Burke and his associates held that the conditions selectively favoring pea root rot were a combination of a low temperature (16-28 °C), exposure of the roots to infested soil, and saturation of the soil after the seedlings had emerged.

SOIL REACTION

There is no convincing evidence either from field observations or laboratory infection studies to support the supposition that pH might be an important factor in pea root rot development. As P. G. Smith and Walker (1941) so adequately demonstrated, *Aphanomyces euteiches* has a remarkable degree of tolerance to pH, showing growth *in vitro* at pH levels between 3.4 and slightly over 8.0. Haenseler (1926), Jones and Linford (1925), and Reinking (1942) concluded that root rot disease incidence, however measured, did not seem to be modified by the soil reaction.

FACTORS OF THE HOST PLANT AND THE INOCULUM

Although the concentration of inoculum (spores) can be reflected in the index of root rot disease severity (Beutz and Lockwood, 1967) the pattern of that severity is not influenced by the number of propagules. In 1965, L. E. Carlson reported a series of tests on excised root tips of peas, using amounts of inoculum ranging from one to 50,000 spores mL⁻¹ of suspending fluid. Although the number of oogonia developed in root sections inoculated with a suspension of 10,000-50,000 spores mL⁻¹ was relatively consistent among replicates, infection was secured even if only a single viable spore was used as inoculum. Although infection by *Aphanomyces euteiches* can be established with a very sparse inoculum load (Yokosawa and Kuninaga, 1977, successfully infected pea seedlings with 50 spores of the fungus g⁻¹ of soil), such is hardly sufficient if cultivar tolerance and susceptibility is to be determined. Small numbers of propagules in the inoculum suspension, Carlson found, yielded mycelium that produced numbers of oogonia in infected roots of tolerant plants that were not much different from oogonial density developed in host tissue of susceptible cultivars. The study by J. L. Lockwood (1960a) and J. L. Lockwood and Ballard (1959) also pointed to a correlation between high disease severity indices and greater (rather than smaller) numbers of inoculating units.

It appears that the age of spores in the inoculum has an influence on pea root rot development, but some of the data on this factor are in conflict. It has been reported by J. L. Lockwood (1960a) that higher disease incidence occurred when seedlings were inoculated with spores from 8- or 11-day-old cultures than from those that were but four days old. Precisely the opposite data appear in the account by J. L. Lockwood and Ballard (1959), but evidently no differences in disease incidence appeared in test plants inoculated with spores two- and 14-hours old. Calculated disease indices were less in test plants inoculated 24 hours prior to being transplanted into sand cultures than in

those exposed to spores of the *Aphanomyces* 12 hours before transplantation (J. L. Lockwood and Ballard, 1959).

What influence does the age of the host plant, or excised root portions of it, have on disease incidence? Haenseler (1925) and Lipman (1925) were the first investigators of pea root rot to suggest that the effect of host age was reflected only in the amount of time intervening between inoculation and the appearance of symptoms: the disease condition simply became evident sooner in younger plants than in older ones even though the ultimate severity of the disease might be the same. According to J. L. Lockwood (1960a) and J. L. Lockwood and Ballard (1959), however, higher disease incidence caused by the *Aphanomyces* could be expected in young pea seedlings (4-6 days) than in older ones (8 days). Data from experimental inoculations carried out by Burke *et al.* (1969b) do not agree, since these investigators found that plants up to 4 days old were less susceptible to *Aphanomyces euteiches* than were those 7 days old. Cho and King (1963), using oospore number (in artificially inoculated, excised pea roots) as the indicator of the degree of infection, counted more of these cells in 7-day-old root segments than in ones either 5 or 23 days old. They reported also that oospores were more prevalent (in artificial inoculation tests) in mature sections of the root (root hair zone) than in the meristematic/elongation region.

PENETRATION

What is known of the pathways of hyphal penetration and the endogenous mycelial development of *Aphanomyces euteiches* in pea roots comes almost exclusively from the experimental work by Cunningham (1961; reported also in Cunningham and Hagedorn, 1962b). Roots of aseptically grown peas were submerged in a spore suspension for 1 ½ -2 hours, and at intervals removed and sectioned. Within two hours after inoculation, simple germ tubes from attached spores had penetrated the epidermis of the root tissue. Entrance was usually intercellular, but occasionally was directly into the host cells. Some appressorium-like structures were produced, but these were uncommon. Eight hours after the root had been inoculated, hyphae of the *Aphanomyces* generally had permeated among the first three cell layers of the cortex, but in some instances grew as deeply into the root as 17 cell layers. Little damage was evident in the invaded cortex save for that concomitant with the cells being forced apart as the hyphae advanced. After 24 hours following inoculation, the mycelium had penetrated to the endodermis, and ramified laterally in the root cortex. By 61 hours, hyphae were present in the phloem and xylem parenchyma, but not in xylem elements. At this time also, the cortex was thoroughly invaded intercellularly (Cunningham, 1961).

Insofar as it was possible to draw conclusions from sectioned root material Cunningham (1961) could not detect any obvious differences in the relative degree to which the roots of a susceptible and a resistant (tolerant?) variety of peas were penetrated. There were, however, slight differences in, for example, the depth to which the mycelium grew into the tissues of the two varietal types (Cunningham and Hagedorn, 1962b). Hyphae were not constricted in their passage through cortical cell

walls. The fungus seldom gained entrance via root hairs, and the magnitude of penetration by its hyphae into the root cap region was less than into the region of cell elongation. While Jones and Drechsler (1925) had maintained that entry by *Aphanomyces euteiches* was accompanied by softening of the invaded tissues, Cunningham and Hagedorn (1962b) did not find this to be the case in their experimental plants.

CHEMOTAXIS

Excised root tips of peas and other susceptible plants were used by Cunningham and Hagedorn (1962a) in experimental work on the attraction of spores of *Aphanomyces euteiches* to the root tissue. Moderate to strong chemotactic response was elicited by root segments of every plant species tested, even those immune to pea root rot (*Zea mays*) and species with very low susceptibility (as determined by artificial inoculation) such as *Lotus corniculatus* L. and *Medicago hispida* Gaertn. Thus, there was no demonstrable correlation between chemotaxis and the degree of host susceptibility or tolerance. For the most part, the region of the root immediately behind the root cap elicited the strongest chemotactic response.

The extent to which planonts of *Aphanomyces euteiches* are attracted to pea roots -- and to germinate at the root surface -- appears to be related to concentration of root exudates (or extract). The higher the concentration of the extract from pea roots, the more intense is planont attraction and subsequent germination (Morrison, 1972). The specific pea cultivar from which the extract is made has no direct influence on the magnitude of chemotaxis.

SURVIVAL AND LONGEVITY OF THE CAUSAL AGENT

It is an established fact that *Aphanomyces euteiches* not only increases and persists in soils regularly planted to peas, it also may survive in the absence of peas during crop rotations only to pounce upon new plantings in some subsequent year. What accounts for this longevity? It is very likely that oospores (Kotova, 1976) guarantee the longevity needed for the fungus to survive prolonged periods away from the safe haven of pea roots, but positive proof is wanting.

The little evidence that is available regarding the capability of the spores of *Aphanomyces euteiches* for prolonged survival is not at all consistent. Hanseler (1925) reported that spores of the fungus retained viability in water for 4-7 days, and in soil for something less than six days (Haenseler, 1926). In nonsterile soil spores remained infective for 20-30 days, although most propagules lost motility within one day, according to Yokosawa and Kuninaga (1977). They found that spores produced from sporangia developed by mycelium in infected roots would survive for 3-4 weeks in soil. Without a doubt it is not the asexual spores that account for the persistence of *A. aphanomyces* in soils for periods of two years (Geach, 1936), 5-6 years (Jones and Drechsler, 1925; Reinking, 1942) or 10-20 years, as H. G. Johnson (1953) reported.

In 1961, Cho reported that after inoculation *Aphanomyces euteiches* successfully invaded a number of wild, weedy plants -- *Chenopodium album* L., *Ambrosia artemisiifolia* L., and *Portulaca oleracea* L., among others -- and he suggested that the fungus might survive by means of invading alternate hosts between pea croppings in fields. Similarly, Carley (1969) concluded from host inoculation studies that alternate hosts in fact "rejuvenated" the fungus by natural passage, and thus served to prolong it in a viable, infective state. Only those crop species ordinarily grown in the field in rotation with peas were susceptible to invasion by *A. euteiches*, hence it was highly probable, Carley proposed, that these plants did in fact aid in maintaining the fungus in field soils.

Experiments by R. T. Sherwood and Hagedorn (1962) demonstrated that oospores of *Aphanomyces euteiches* survived burial for two years in saturated soils, those held at 25% of saturation, and in air-dry soils. These cells even withstood alternate drying and freezing, or continuous freezing over a two-year period. It was also shown that *A. euteiches* could grow saprotrophically in soil, but could not traverse untreated soil by means of hyphae. In addition, there was evidence that the fungus invaded and colonized plant debris. However, Sherwood and Hagedorn did not believe that the ability of *A. euteiches* to live as a saprotroph contributed to its longevity in the soil.

In sum, it is clear that there is ample evidence for oospore durability as one of the chief factors permitting the causal agent to persist in infested fields. These cells even survive passage through snails (Bhalla and Mitchell, 1970), an event further attesting to their hardiness. In addition, it seems likely that dissemination of the *Aphanomyces* must involve its oospores to some extent. Jones and Drechsler (1925) thought that any agent capable of transferring soil from one field to another also could move the fungus: wind and the flow of surface water, to name two such agents. They did not believe that the fungus was introduced into localities by infected seeds. It is of course possible that infected roots themselves are a source of inoculum through the medium of spores. The only direct evidence at hand for this supposition is that provided by Yokosawa and Kuninaga (1977). They calculated that *A. euteiches* in infected roots in soil at 75-90% of its water holding capacity produced 9×10^4 spores; at the 50-60% level, the endogenous mycelium and sporangia were capable of producing 1.8×10^5 spores.

THE PHYSIOLOGY OF PARASITISM

Studies on root rot of peas are not limited to epidemiology in the classical sense. Some investigators have sought information on infectivity and disease severity through experimentation with physiological and biochemical parameters.

In a general way, Haglund (1960) studied the gross chemical nature of certain tissues of *Pisum sativum* to determine if nutrients in those tissues were in some way related to pathogenicity. He found that components of susceptible varieties of peas supported more growth of *Aphanomyces euteiches* than did those of resistant lines. Moreover, the more virulent isolates of the fungus grew better on excised pea seed tissue than did the weakly pathogenic ones.

As part of an extensive exploration into possible influences of culture conditions on *Aphanomyces* root rot of peas, P. G. Smith and Walker (1941) experimented with nutrient solutions. They grew pea seedlings in sand culture, inoculated the roots with spore suspensions of the causal agent, and then added to the sand various nutrients. If the concentration of the nutrient solution was increased 4-5 times over that used in the control pots, no root rot developed, yet modifications in the ratio of the three basic elements N:P:K did not reduce the incidence of the disease when it occurred in test plants. To explain the disease-suppressing quality of a highly concentrated mineral nutrient solution, Smith and Walker suggested that at elevated levels of salts in the surrounding medium the roots become woody (more so than at low levels), and thus mechanically prevented germ tube penetration. Alternative explanations, of course, come quickly to mind.

Sulfur containing compounds have an effect on disease development and severity (Papavizas and Davey, 1963a) as do certain amino acids and related compounds (Papavizas and Davey, 1963b). Moreover, particular sulfur compounds (Papavizas and Davey, 1960a, b) influence growth (Chapter 17) and reproduction of *Aphanomyces euteiches*.

Inoculated pea seedlings propagated in nutrient solutions containing various test compounds, were used by Papavizas and Davey in their extensive physiological work on the disease and its causal agent. Root rot was severe (as measured by scaled disease ratings and estimates of oospore abundance in infected tissue) in the presence of oxidized sulfur (+4 or +6) and reduced inorganic and organic sulfur as well. While DL-methionine supported both vegetative growth and reproduction of *Aphanomyces euteiches in vitro* (Papavizas and Davey, 1960a, b), the amino acid (with its sulfur side chain) prevented disease development in susceptible pea seedlings. The L-isomer of methionine also was more effective in suppressing pathogenesis than was the D-isomer. In addition, the methionine-related compounds S-methyl-L-cysteine, DL-ethionine, and DL-isopropionine prevented root rot in laboratory culture, but DL-cystine·2 HCl and L-cysteine did not. Preconditioning the pea seedlings by growing them in a nutrient solution containing methionine did not prevent the disease, but did reduce its severity. While the oxidation state of sulfur (Papavizas and Davey, 1963a) could affect *in vitro* mycelium production, sporulation, penetration, and infection were not influenced measurably.

The source of nitrogen has an effect on the severity of pea root rot, if one may judge from the results of Carley's (1969) experimental work with greenhouse-grown seedlings. *Aphanomyces*-incited root rot was more severe when ammonium nitrogen was available to the host plant than when nitrate nitrogen only was present. He attributed this response to an influence of nitrogen on the invading fungus rather than on the host itself.

Of 60 amino compounds tested by Papavizas and Davey (1963b), only six were effective in preventing pea root rot from developing in inoculated roots of susceptible seedlings. Fourteen of the chemicals were partially operative in modifying disease severity, but the remainder were ineffective in this regard. All of the compounds that

showed some degree of disease suppression ability had CH₃, NH₂, or COOH groups in the molecule. Excerpts from the data recorded by Papavizas and Davey are given in Table 43. Obviously, there is a high degree of specificity among the various sulfur-free compounds in their ability to prevent or suppress a pathogenic response in inoculated peas. When diseased pea plants are exposed to certain amino acids (glutamic and aspartic acid, for example) after having been treated with β-methylaspartic acid, the inhibitory effect of the latter is nullified (Lumsden *et al.*, 1970).

Ayers and Papavizas (1965) demonstrated that *Aphanomyces euteiches* produced pectin depolymerase that was recoverable from culture filtrates. The fungus apparently was unable to synthesize pectin methylesterase, but infected and noninfected pea roots did so (confirmed by Temp, 1966). The investigators suggested that the ability of methionine and norleucine, for example, to confer on pea roots resistance to the *Aphanomyces* might involve increased methylation of host pectin, making it less susceptible to pectin-degrading enzymes. Further analyses of the endopolygalacturonase (Ayers and Papavizas, 1965, had suggested that the pectolytic enzyme in *A. euteiches* was not classical polygalacturonase), and its purification were reported by Ayers *et al.*, in 1969(a, b). Endopolygalacturonase possibly is responsible for the softening effect that is characteristic of pea root tissue invaded by *A. euteiches* (Ayers *et al.*, 1969b). Lumsden *et al.* (1970) believed that the effect of β-methylaspartic acid in preventing root rot in experimentally inoculated peas was by its action on the fungus rather than on the host (through methylation of pectic substances as Ayers and Papavizas had proposed).

In a previous chapter (17), the effect of various phytoalexins on the growth and development of saprolegniaceous fungi was considered. One of these agents is pisatin, investigated by Pueppke and VanEtten (1974, 1976), VanEtten (1973, 1976), and VanEtten and Pueppke (1976). Pisatin also has been studied for its role in the process of pathogenesis caused by *Aphanomyces euteiches* and it is known (communication) that this species is capable of degrading pisatin (at least to the metabolite 6a-hydroxy-inermin).

By puncturing the epicotyl of a pea seedling and placing over the wound a mycelium plug of *Aphanomyces euteiches*, Pueppke and Van Etten (1974) were able to induce the formation of a single lesion. Subsequently, they extracted pisatin (identified by thin layer chromatography) from the lesion. Thirty-six hours after inoculation of a susceptible seedling epicotyl and challenge with the fungus, the concentration of pisatin in the infected tissue was eight times greater than necessary for preventing the growth of *A. euteiches in vitro*. However, the accumulation of pisatin in the epicotyl did not prevent the *Aphanomyces* from growing into the root system (oospores in the cortical tissue ten days after inoculation). Histological sections showed that the fungus was essentially restricted to the lesion, although in one instance Pueppke and VanEtten (1976) detected hyphae in advance of the margin. Even though there was a high concentration of the phytoalexin in the necrotic area, the lesion continued to enlarge. In fact, if pea epicotyls were stimulated by the application of ultraviolet light to form pisatin and then were inoculated, lesions developed. In better than 75% of the attempts at isolation *A. euteiches* it was recovered from the lesion margin and proximal to the

puncture wound at the site of inoculation. Pueppke and VanEtten (1976) concluded that their data did not support the view of a compatible pathogen overcoming the defense action of phytoalexin, and being able to induce the disease it was capable of inciting.

The precise role of the isoflavonoid phytoalexins (Deverall, 1972a, b; Kuć 1972; K. O. Müller, 1961; Pueppke, 1978; Wit-Elshove, 1968, 1969) remains obscure, although they are undeniably associated with pathological conditions in two principal ways. First, the phytoalexins appear to be correlated, in a time-frame reference, with the restriction of a pathogen in host tissue. Secondly, treatment that suppresses these compounds prior to inoculation can result in a resistant reaction developing into a susceptible one (VanEtten and Pueppke, 1976).

RACES AND RESISTANCE

In their account of *Aphanomyces euteiches* Jones and Drechsler (1925) remarked that some foreign pea variety introductions were tolerant of the fungus, but none was resistant. Jones (1926) observed that varieties of *Pisum sativum* which were resistant in some field plots, for instance, were susceptible in others, and concluded that resistance to the *Aphanomyces* was only a minor factor. He contended that the plant's ability to escape secondary invaders was a major factor in its tolerance of root rot. From a study of some 45 pea varieties, Labrousse (1933) found both resistant and susceptible varieties. J. L. Lockwood (1960b) and J. L. Lockwood and Ballard (1960) tested 805 introductions finding that none was highly resistant or immune. Breeding to reduce susceptibility was not effective.

Various experiments designed to determine what factors -- those inherent in the nature of host or pathogen and those dictated by environment -- influence resistance or susceptibility to pea root rot have been carried out. J. L. Lockwood and Ballard (1959) set out to uncover the role of 12 experimental variables on susceptibility of pea seedlings, among them distance of inoculum from the host, seedling age, water level before and after inoculation, and the volume and age of the inoculum. Ten of the variables had some effect as expressed in the resulting disease index. They found (J. L. Lockwood and Ballard, 1959, 1960), for example, that a single inoculation of hosts with the fungus was sufficient to establish the disease; the age of the spores used as inoculum had no effect on disease severity, and after inoculation, soil (sand) saturation fostered severe disease expression. Lowered soil temperatures resulted in suppressed disease severity. Cunningham (1961) reported that the percentage of germinated spores of *Aphanomyces euteiches* was greater on susceptible pea roots than on tolerant ones, and suggested that such factors as fungal strain differences or nature of the inoculum could influence susceptibility/resistance trials.

A few attempts have been made to standardize the testing approach for determining resistance in peas. Shehata and associates (1976) devised an environmental shift technique. By changing the external conditions during testing of inoculated peas in environmental control chambers and the greenhouse, they stimulated a resistant

cultivar to survive heavy inoculation and susceptible ones to overcome infection when the conditions were changed to favor the host plant. Their method separated resistant from susceptible cultivars based upon the time of the plant's death after inoculation. Shehata, Grau *et al.* (1976) also reported that in some instances pea varieties could be evaluated for simultaneous response to *Aphanomyces euteiches* and *Fusarium oxysporum* f. sp. *pisi*. It was possible to assay pea cultivars, King and Cho (1962) reported, by inoculating excised root segments from various cultivars and subsequently evaluating their resistance or susceptibility on the basis of numbers of oogonia produced in the root pieces. There were fewer oogonia in resistant lines than in susceptible commercial introductions. Carlson (1965) tested 14 pea varieties for resistance by the excised root tip method, and also concluded that it was a suitable method by which to obtain data on this aspect of pea root rot.

The experimental breeding program conducted by Marx and his collaborators (1972) provided some data on the inheritance of resistance in peas. They used a consistently superior line (in terms of apparent resistance to the causal agent) and bred it with other cultivars. Progeny tests indicated that most individuals retained a relatively constant level of tolerance to *Aphanomyces euteiches*, indicating that some degree of genetic improvement had been attained. None of the progeny, however, attained the degree of tolerance expressed by the parental line. The investigators concluded that tolerance was associated with dominant wild type alleles at three unlinked loci.

When excised root tips were exposed to spores of various isolates of *Aphanomyces euteiches* Carlson (1965) found that there were measurable differences in the numbers of oogonia produced in those root segments; this indicated that strains of the fungus existed. Although among the 14 isolates used by Beute and Lockwood (1967) in resistance testing, there were substantial variations in pathogenicity expressed by inoculated pea plants; the patterns of disease severity were similar with one exception. These two investigators concluded that the one isolate inducing the observed variant in disease pattern was a different pathogenic race than the remaining 13 isolates. In this case, then, virulence was not the measure of strain or race identification.

The results of a series of inoculations on various pea varieties led Sundheim (1972) to the view that there were race differences (expressed by the number of dead plants ten days after inoculation) among 14 test isolates, but none was identical to the "race" of the culture that Beute and Lockwood (1967) had singled out from the series of strains they studied. Later, however, among additional isolations of *Aphanomyces euteiches* from Norwegian soils, Sundheim and Wiggen (1972) identified five physiological races, one of which was similar to that reported by Beute and Lockwood. There was no indication of any geographical distribution differences among the isolates in Norway.

Varieties of *Phaseolus vulgaris* L., *P. lunatus* L., and *P. coccineus* L. are known to exhibit differential susceptibility to invasion by *Aphanomyces euteiches*. Using selections of these species in a series of experimental inoculations Carley (1970) discovered what he believed to be seven races of the fungus among eight test isolates. Solberg (1925)

recovered *A. euteiches* from roots of a number of plants including beans, sweet pea, *Orobus vernus*, and *Caragana arborescens*, among others. She believed that each of these isolates represented races of the fungus, but there are no supportive experimental data in her account.

HOST RANGE

In their initial account of *Aphanomyces euteiches* as the cause of pea root rot, Jones and Drechsler (1925) stated that no varieties of *Pisum sativum* or *P. sativum* subsp. *arvense* (L.) Poir. were immune to the fungus, although some showed resistance. Since that time, eighty or more species or varieties of plants have been reported as hosts for this fungus (Carley, 1969; L. E. Carlson, 1965; Haenseler, 1925, 1926; Linford, 1927; R. T. Sherwood, 1958; Solberg, 1925; Lipman, 1926; Geach, 1936; R. T. Sherwood and Hagedorn, 1962; Walker and Hare, 1943). Not only has the fungus been reported from common field plants and weedy species, it has been isolated from (or presumably collected in) such species as *Echinodorus brevipedicellatus* Buch (Ridings and Zettler, 1973), *Apium graveolens* L. (Doran *et al.*, 1942), and, among the conifers, *Picea engelmannii* Parry ex Engelm., *Pinus banksiana* Lamb, and *Pseudotsuga menziesii* (Mirb.) Franco (Eliason, 1928). *Aphanomyces euteiches* has been reported to infect members of 15 genera of Leguminosae, in addition to garden and field peas. A few examples illustrate the breadth of the presumed host range in this one family: *Cicer arietinum* L., *Glycine max* (L.) Merr., *Lathyrus odoratus* L., *Lupinus luteus* L., *Medicago sativa* L., *Melilotus alba* Desr., *Onobrychia viciifolia* Scop., *Phaseolus aureus* Roxb., *P. lunatus* L., *Trifolium pratense* L., *Vicia dasycarpa* Ten., *V. faba* L., *V. gigantea* Hook., *V. pannonica* Crantz, *V. sativa* L., and *V. angustifolia* (L.) Walp. Other plant families with representative species said to be hosts for *A. euteiches* are recorded in Table 44; the comprehensive host list reported by Papavizas and Ayers (1974) may be consulted for additional species.

With few exceptions, the very liberal host range of *Aphanomyces euteiches* has been determined from the results of inoculations of whole plants (Eliason, 1928; R. T. Sherwood and Hagedorn, 1962, for example), or excised roots (such reports as those by L. E. Carlson, 1965, and Carley, 1969) with pure cultures of the fungus, or from observations on plants grown in nonsterile soil (Geach, 1936, for instance). Other hosts have been singled out by artificially inoculating greenhouse-grown plants (Haenseler, 1926), and even by observation of oogonia in the dead roots of plants (Lipman, 1926). It should not be surprising, then, that agreement on all reported segments of the host range is lacking. Buisman's (1927) finding of *A. euteiches* on *Viola* species in Holland was questioned by Drechsler (1954b); R. T. Sherwood (1958) was unable to infect artificially such common plants as celery, corn, barley, wheat, and cucumber -- all reported at various times by various workers as hosts for this *Aphanomyces*. To be sure, Linford (1927), for example, found oospores of a fungus he identified as *A. euteiches* in the roots of field-grown *Medicago sativa* and *Melilotus officinalis* (L.) Lam., but did not detect these structures in field-grown plants of *Melilotus alba*, *Trifolium hybridum* L., or *T. pratense* L. Such direct observations seemingly have been rare as the search for other hosts for *A. euteiches* has progressed.

Having found oospores (presumably of *Aphanomyces euteiches*) in the roots of plants grown in nonsterile soil in the greenhouse, Linford (1927) reported a number of species of legumes to be susceptible to the fungus. However, R. T. Sherwood (1958) very cogently has remarked that there is great uncertainty about a host range determined solely from microscopic detection of oospores in root tissue. A host listing established on the basis of infections developed in plants grown in natural, untreated and unsterilized soil (Linford, 1927) also is of questionable validity. Moreover, results from the use of excised root sections in laboratory tests to determine host range (Carley, 1969; Carlson, 1965) at best may only border on reality.

The very extensive host range of *Aphanomyces euteiches* must be viewed as an approximation -- as Papavizas and Ayers (1974) have done -- since so few determinations have been confirmed by field observations, isolations, and reinoculations in surroundings approaching conditions in which root rot develops naturally. Artificial inoculations in the greenhouse or laboratory surely must confer on the pathogen an undeserved advantage and impart to it a benefit that it cannot enjoy in the harsh environment of the field. But perhaps *A. euteiches* truly is an omnivorous facultative pathogen as the host range would seem to suggest!

INTERACTIONS

Aphanomyces euteiches is not without its antagonists or benefactors, but the actual impact of living elements (and viruses) on its development is far from thoroughly analyzed. The earliest report of an antagonist to *A. euteiches* seems to be that by Drechsler (1938). He found that haustoria from the hyphomycete *Dactyella spermatophaga* Dreschler penetrated the oospores of the watermold, *in vitro*, and destroyed the contents. Over a period of time, he suggested, such attacks on the oospores of root-rotting fungi might promote soil sanitation.

Additional instances of the parasitism of *Aphanomyces euteiches* by other fungi are known. *Hyphochytrium catenoides* Karling is one such invader, and possibly could be thought of as an agent involved in the decline of populations of Oomycetes in the soil (Ayers and Lumsden, 1977). Oospores from *A. euteiches* were harvested by Sneh and his collaborators (1977), then exposed to soil samples, and were subsequently (4-10 days incubation) found to be infected by chytridiaceous fungi (not identified). Species of *Pythium*, Alconero and Hagedorn (1967) found, did not predispose roots to invasion by the pea root rot fungus, nor did they to any extent influence its infectivity.

Whether or not organisms other than fungi influence the pathogenic inclinations of *Aphanomyces euteiches* also has been explored. When two species of nematode were mixed into soil with inoculum of *A. euteiches* and susceptible seedlings transplanted into that soil, Haglund and King (1961a) noticed an amplification (over controls) in root rot severity. Moreover, severity increased as the concentration of nematodes was raised. They postulated that the nematodes either functioned to alter the host tissue's physiology (and thus make it more readily available to the fungus) or to create avenues of entry. Oyekan and Mitchell (1972) came upon essentially the same result in

experiments with populations of *Pratylenchus penetrans* and two soil types. They discovered, however, that if very high inoculum levels of *A. euteiches* were used, any effect of the nematode (predisposing the tissue to invasion) was counteracted. The survey by Temp and Hagedorn (1968) of Wisconsin fields known to harbor *A. euteiches*, when coupled with an analysis of nematode populations, did not expose any relationships. Haglund and King (1959) mention the interaction of nematodes and pea root rot, but do not name the fungus involved.

It has been demonstrated (J. L. Lockwood, 1959) that species of *Streptomyces* in soil appeared to be responsible for the lysis of *Aphanomyces euteiches* mycelium buried in soil samples. By means of a plate layering technique Carley (1969) discovered that the number of bacteria alleged to be antagonistic to *A. euteiches* increased when nitrate nitrogen was available to the fungus but not when ammonium nitrogen was supplied. While Carley's tests were far from conclusive, he suggested that the reason nitrate nitrogen appeared to suppress pea root rot was because it favored the growth of microorganisms antagonistic to the causal fungus. Cho (1961) had noticed earlier that two species of bacteria in excised root tip cultures seemed to promote infection of the root tissue by *A. euteiches*. Two other species of bacteria, however, appeared to suppress infection.

Farley and Lockwood (1964) inoculated a number of pea seedlings with *Aphanomyces euteiches* alone, and others with the fungus followed by exposure of those seedlings to one of four viruses: pea mosaic, alfalfa mosaic, pea enation, and bean yellow mosaic (infested plant sap was rubbed onto the leaves). In every case, the disease severity index of pea root rot was higher in the dual-inoculated plants than in those subjected to the fungus alone. To explain the mechanism whereby virus-infected peas were more severely diseased by *A. euteiches* than virus-free ones, Beute and Lockwood (1968) compared the nature of root exudates from virus-infected and virus-free plants. During the period when root exudation from plants harboring virus was increasing, the severity of root rot also rose. The exudates contained various carbohydrates, amino acids, electrolytes, and nucleotides, and when pea plants that had been inoculated with *A. euteiches* were exposed to a comparable mixture of amino acids, root rot severity was elevated substantially. Beute and Lockwood proposed that the virus infection increased root permeability and compounds released (in the exudate) as a result increased the inoculum potential of the pathogen.

CONTROL AND SUPPRESSION

The control of pea root rot -- its management as some would have it -- is of singular importance in the commercial production of peas, and it follows that *Aphanomyces euteiches* has been subjected to all manner of practices, some gentle, some severe, in attempts to reduce its damaging effect. Control methods are quite properly an essential part of pathology, but for purposes of this mycologically-flavored account need not be treated in detail. We therefore confine our remarks largely to listing some general methods that have been tested or utilized, and by whom. Experiment station

bulletin reports, recommendations, or analyses of control practices, and accounts in which *A. euteiches* is not mentioned by name (Pfleger *et al.*, 1976), are ignored, as are all published abstracts on control. The review by Papavizas and Ayres (1974) makes mention of some of these accounts.

MINERAL FERTILIZATION

One of the earliest practices to be recommended for the control of pea root rot was the heavy application of fertilizers. Although this proved to be a beneficial method, reducing but not eliminating the disease (Walker and Snyder, 1933), it on occasion had injurious side effects, particularly if simple inorganic nitrogen salts were used. Moreover, the suppression of root rot by fertilizing was found to be effective only at the beginning of the growing season. This is not, accordingly, a method of widespread use. See: Davey and Papavizas (1961), Geach (1936), Haenseler (1926), H. G. Johnson (1953), Schroeder and Reinking (1947), P. G. Smith and Walker (1941), Wade (1955), Walker and Hare (1943), Walker and Musbach (1939), Walker and Snyder (1933).

MINERAL AND METALLIC SALTS

Copper compounds are effective agents to suppress the severity index of pea root rot (J. A. Lewis, 1973a). Aluminum, calcium, barium, and zinc salts also are adequate suppressants, but combinations of compounds containing some of these ions must be incorporated with copper salts to realize the most drastic reduction in the disease (J. A. Lewis, 1973b). An extensive test of the efficacy of various calcium compounds (gypsum, calcitic and dolomitic limestone, among others) was reported by J. A. Lewis, in 1977. In greenhouse experiments CaSO_4 , CaSO_3 , CaO , and $\text{Ca}(\text{OH})_2$ reduced pea root rot, but calcium phosphate did not. Both hydrated lime and calcitic limestone lowered the prevalence of the disease in field plots artificially infested with *Aphanomyces euteiches*. Groth and his associates (1979), to the contrary, found that CaCO_3 (calcitic limestone) did not suppress the disease caused by this fungus in planted fields displaying either moderate or severe pea root rot.

FUNGICIDES, HERBICIDES, AND FUMIGANTS

Of the many fungicides tested for their ability to reduce pea root rot, Dexon (sodium *p*-dimethylaminobenzenediazo sodium sulfonate) is the leading candidate (Alconero and Hagedorn, 1968; Grau, 1975; J. E. Mitchell and Hagedorn, 1966, 1969, 1971; Papavizas, 1967; Pivaral, 1967). Among other chemicals tested for control qualities are the dinitroanilines (trifluralin and dinitramine) and dinitrophenols (Dinoseb) calcium cyanamide; fumigants such as vapors from carbon disulfide, dimethyl sulfide, and methanethiol; 1, 2-dichloro-1-(methylsulfonyl) ethylene; Zineb; Benomyl; amidosulfonylmethyl-phenylthioether, and, as a seed protectant, Spergon

(Grau, 1975, 1977; Grau and Reiling, 1977; Haenseler and Moyer, 1937; R. G. Harvey *et al.*, 1975; J. A. Lewis and Papavizas, 1971a; J. L. Lockwood, 1961; Olofsson, 1967; Reinking and Newhall, 1950; Sacher *et al.*, 1977, 1978; Sharvelle *et al.*, 1942; Sundheim, 1971; Teasdale *et al.*, 1978). The effectiveness of the several antifungal agents varies as a function of a number of factors such as time and method of application, concentration, and soil conditions. Some agents are known to be effective only in greenhouse testing, and others suppress the fungus in culture, but not *in vivo*. Finally, it must be recognized that a few agents appear to delay the inception of root rot in fields, but in doing so may "allow" the plants to gain vigorous growth so that in spite of subsequent infection, some yield can be realized. In this connection, Katan and Eshel (1973) conclude that herbicides may induce changes in disease incidence and severity by affecting one (or perhaps more) of the three elements in a pathological condition: the host, the pathogen, or the microorganisms in the environment. The relative effectiveness of some nonvolatile fungicides, fertilizers, and nitrogen sources in reducing root rot in susceptible peas planted in *Aphanomyces*-infested soil is shown in Figure 56.

SULFUR AND NONSULFUR COMPOUNDS

Papavizas and Davey (1960a, b) demonstrated that certain amino acids and related organic sulfur compounds stimulated the growth of *Aphanomyces euteiches*. Later (Papavizas and Davey, 1963a, b) it was shown that particular sulfur-containing amino acids, notably DL-methionine (Fig. 57), had some chemotherapeutic effect, at least in laboratory and greenhouse conditions (Andel, 1966). Considerable experimental work on reducing the growth of *A. euteiches* has been done basically with two groups of amino acids (naturally-occurring, generally *l*-acids, and stereoisomers) and certain amino acid analogues not found in plants (Davey and Papavizas, 1963; Lumsden *et al.*, 1970; J. E. Mitchell and Hagedorn, 1966; Papavizas, 1964, 1967; Papavizas and Davey, 1962). Not all data, however, support the view that methionine is an efficacious agent. J. E. Mitchell and Hagedorn (1966) found this organic sulfur compound to be ineffective both in greenhouse and field-testing. It is in any case true that amino compounds probably are impractical for general use in field control practices.

ORGANIC AMENDMENTS

Both cruciferous plant parts (dried or fresh portions of such species as *Brassica oleracea* var. *capitata* L., *B. oleracea* var. *gemnifera* DC, *B. rapa* L., and *Raphanus sativa* L., for instance) and non-cruciferous parts [*Zea mays* L., *Avena sativa* L., and *Glycine max* L., among others] as well as water extracts and vapors from decomposing leaves have been used as soil treatments in attempts to reduce root rot caused by *Aphanomyces euteiches*. Amendments using leaves of *Brassica* species are known to be effective in greenhouse tests, but not (J. E. Mitchell and Hagedorn, 1969) in field applications (the level of application perhaps was insufficient). Consult papers by Davey and Papavizas (1961),

J. A. Lewis and Papavizas (1970), Papavizas (1966, 1967), Papavizas and Lewis (1970, 1971), and R. T. Sherwood and Hagedorn (1962). The simple addition of such materials as chopped corn stalks or dry straw of rye does not reduce root rot incidence, but rye plants used as green manure suppresses the disease to some extent (H. G. Johnson, 1953).

CROP SEQUENCES (ROTATION)

Almost from the beginning of serious study of pea root rot, investigators agreed that the disease could be expected to be severe in fields planted regularly to peas, and that crop rotation was beneficial in reducing disease incidence. The first well-designed study of the value of cropping practices in the control of pea root rot was that by Temp and Hagedorn (1967). They demonstrated a strong positive relationship between the number of pea crops in a field and disease incidence. A rotation providing three or four years of other crops between pea plantings did not reduce substantially the root rot potential of those fields, and high disease indices were realized in fields planted to peas after a 6-8 year lapse. The crops used in rotation with peas also are of singular importance. KenKnight (1944) concluded that because sweetclover (*Melilotus* spp.), alfalfa (*Medicago sativa*) and vetch (*Vicia* spp.) were susceptible to *Aphanomyces euteiches* they should not precede peas in any crop rotation scheme.

The longevity of oospores of *Aphanomyces euteiches* in soil, and the ability of the fungus to occupy (in field soil remnants of plant hosts other than peas would seem to suggest that very long rotations (ten years or more) are necessary to reduce field-borne inoculum to such a degree that pea plantings would give a profitable yield. Information on the efficacy of crop rotation in the control of *A. euteiches* is reasonably extensive: Drechsler (1925), Harter *et al.* (1935), H. G. Johnson (1953), Jones and Drechsler (1925), Jones and Linford (1925), KenKnight (1944), Linford and Vaughn (1925), Reinking (1942), Schroeder and Reinking (1947), Walker and Hare (1943), Zogg (1964).

It is possible to determine reasonably accurately the root rot potential of fields (*see* next section). Such information, if applied accurately with foreknowledge of the factors detrimental to the causal organism and disease inception and development, can be useful to some degree in avoiding the malady. This lends weight to the suggestion by R. T. Sherwood and Hagedorn (1958) that withholding pea plantings from fields known to have high root rot potentials is the only practical method of control.

BIOLOGICAL METHODS

Studies by Kommedahl and Windels (1978) and Windels and Kommedahl (1978) constitute the chief efforts directed specifically at suppressing pea root rot by the effect of interacting or antagonist microorganisms on the causal agent. Fifty-nine microorganisms isolated from roots and seeds of pea were tested for antagonism to *Aphanomyces euteiches*. In experiments conducted with agar media, only one isolate, a

bacterium, inhibited two isolates of the fungus. Field tests of antagonist-treated seeds proved to be inconclusive, although some of the fungi and bacteria used were associated with improved field stands of pea. When applied as conidia to the seed coat of peas, *Penicillium oxalicum* Currie and Thom was more effective (Windels and Kommedahl, 1978) in preventing pre- than postemergence damping off due to root pathogens (including the *Aphanomyces*).

METHODOLOGY

In the foregoing account we have referred without explanation to such aspects of the pathology of pea root rot as tolerance and indexing. These are respectable methods of measuring aspects of the disease, and, together with a few other special techniques, warrant attention. We have already (Chapter 2) chronicled the culture methods developed for inducing planont formation in *Aphanomyces euteiches* (Hoch and Mitchell, 1972c), and have noted that Bhalla and Mitchell (1970) secured mycelium-free oospores of the fungus by passage through snails.

Boosalis and Scharen (1959), recognizing the need for a rapid (and successful) means of detecting *Aphanomyces euteiches* and other pea root pathogens in plant debris, developed a method for direct microscopic examination. A soil sample containing plant fragments is suspended in water, allowed to settle, and the supernatant screened. This process is repeated, and the residue subsequently comminuted through a 200-mesh screen. The residue plant material on the screen then is put into water, and the fragments examined microscopically for oospores.

To provide a reliable test for the tolerance of pea varieties and cultivars to *Aphanomyces euteiches*, Haglund and King (1961b) attempted to develop a standard inoculation method. Their technique consisted of inoculating three varieties of peas (one resistant, one susceptible, and one moderately resistant) with spore suspensions of 25,000, 50,000, and 100,000 per plant. The method gave uniform results only for the very virulent strains of the fungus. Another seedling test method was devised by J. E. Mitchell and his collaborators (1969). Pea seeds were germinated in vermiculite, then removed and placed on paper toweling. Soil debris was scattered over a given area on the root, and the seeding then rolled up in the towel ("rolled tube"). The plants then were incubated, with whatever propagules were extant in the soil providing the inoculum. Screened soil and sedimented particulate matter could be tested in this fashion. Estimations from the results of a series of tests of the method indicated that if 25% or more of the seedlings became infected the soil used as the inoculum source came from fields with severe root rot potential (J. E. Mitchell *et al.*, 1969). Moreover, the technique was alleged to be sensitive enough to reflect the type of propagule in the soil; if seedlings became infected in a short period of time, 3-5 days, for instance, it could be assumed that spores or mycelium were the propagules. Two-fold differences in the amount of inoculum in the soil could be detected by this rolled tube technique.

The use of the excised root tip method for pea root rot analysis (L. E. Carlson,

1965; Carley, 1969) is of course justified only if its limitations and specific application can be defined rather precisely. Morrison *et al.* (1971) attempted to determine some of the factors influencing the reliability of the technique. They used motile spores of *Aphanomyces euteiches* as inoculum, and counted the numbers of oogonia (oospores) in the root sections following an incubation period. To obtain results within the 95% confidence limits, a minimum test sample size of 40 root tips was necessary. Certain variables -- not unsurprisingly -- attended the method. There was more rapid development of the infection (and subsequent disease) in injured (cut) roots than in intact ones, and both spore motility and incidence of contamination (as well as the resistance or susceptibility of the pea varieties themselves) influenced the results. In addition, the concentration of spores in the inoculum was very important: more spores were needed to induce infection in resistant than in susceptible lines. Morrison and his colleagues concluded that the method was reliable, that is, the results of tests were reproducible, provided conditions in the test procedure were controlled carefully.

Some system of rating the magnitude of *Aphanomyces* root rot in experimental work on greenhouse-grown peas and in field surveys is essential for the proper analysis of the disease. The disease severity index (DSI) devised by P. G. Smith and Walker (1941) has enjoyed most widespread use. On the basis of this index pea plants are individually rated on the degree of infection as expressed by symptoms: 0 = no symptoms; 1 = slight water-soaked appearance of the epicotyl or of the primary or secondary roots; 2 = moderate water-soaked expression; 3 = softening of the infected area, but no visible degeneration, and 4 = discoloration, accompanied by the collapse of tissues (this disease class includes dead plants). After all plants are rated as to disease class, a formula is used to calculate the disease severity index:

$$\text{DSI} = \frac{\text{Sum of disease class (0-4) x number of plants in that class}}{\text{Total number of plants}}$$

A DSI of 0 would indicate that all plants in the experimental lots were healthy after inoculation and incubation, and if all plants were rated as class 4, the severity index would be 100. A somewhat different method was devised by R. T. Sherwood and Hagedorn (1958) namely, the calculation of a field disease severity index based on four classes of infection (none, slight, moderate, severe). DSI calculations were made from infection experiments with pea plants grown in soil. The potting soil consisted of samples from pea fields in which the DSI had been determined previously. There was a definite correlation between the greenhouse index and the severity in the field, and the investigators concluded accordingly that greenhouse indexing provided a reliable clue to the potential for root rot in field-grown peas. The field index (R. T. Sherwood, 1958) was determined by this formula:

$$\text{Field Index} = \frac{\text{Sum of the total field area having plants of a given class} \times \text{Class value}}{3}$$

Obviously, unpredictable field factors -- variable precipitation, seedling rate, soil fertility, and weed competition, among others -- reduce the degree of correlation between greenhouse indexing (the estimate of expected severity in the field) and the actual magnitude of severity of root rot that could develop in planted fields. Reiling and his associates (1960) confirmed the relatedness between the results of greenhouse indexing and potential pea root rot, but did not refer to any specific causal organism. The work of Olofsson (1967), in Sweden, on pea root rot substantiated the hypothesis proposed by Sherwood and Hagedorn.