

CHAPTER 28. Parasites and Pathogens: Blackroot of Sugar Beets, and Other Diseases

BLACKROOT (*Aphanomyces cochlioides*)

Beta vulgaris L. (sugar beet, table beet, mangel) is subject to a disease known as blackroot. Buchholtz and Meredith (1944:488) refer to the condition as tiprot, and earlier, Murphy (1927) called it rootburn of beets. Both Byford and Prince (1976) and W. C. Moore (1959) discussed the disease as "black leg." In 1949(b) Ernould demonstrated that blackroot was not the same pathological condition as that known as strangling disease of beets.

SYMPTOMATOLOGY AND THE CAUSAL AGENT

Blackroot is known throughout the beet growing regions of the Old and New World, although its incidence apparently is rather varied (Kühnel, 1978). At certain early stages in its inception, the disease can be distinguished only with great difficulty from sugar beet root rot caused by *Phoma betae* (Oud.) Frank, *Rhizoctonia solani* Kuehn, *Pellicularia filamentosa* (Pat.) Rogers, and species of *Pythium*.

Symptoms of blackroot caused by *Aphanomyces cochlioides* are expressed in two phases of the disease, an early one, the acute phase, and a later, chronic one, of longer duration. In the acute phase, the beet seedlings may suffer pre- or postemergence damping off. The seedling's hypocotyl shows evidence of infection at the ground line, and a water soaked symptom extends along the hypocotyl and into the young taproot. In time, the petioles of the first leaves also may show water soaking. Eventually the taproot and hypocotyl become brownish, then jet black as infection proceeds. The hypocotyl shrinks to a collapsed, blackened, thread-like strand (Buchholtz and Meredith, 1944; Campbell, 1939; Coons *et al.*, 1946; Drechsler, 1929; Henderson and Bochstahler, 1946; W. E. McKeen, 1949; Papavizas and Ayres, 1974; Warren, 1948). Nuckols and Tompkins (1929) reported that one characteristic symptom of plants infected by blackroot is a distinctively bowed condition developing in the petioles.

The chronic phase of blackroot -- developed in older plants that have escaped or survived the acute or seedling phase -- is recognized externally by the stunted appearance of the host, accompanied by chlorosis in the lower leaves. Infected taproot tissues in plants having the chronic type of infection are initially greenish-yellow, but subsequently become blackened. Upon drying, the infected area shrivels into strands of vascular tissue. Severe wilting is also a chronic phase symptom of infected beets in the field and greenhouse. This advanced stage of the disease of course reduces yield as well as the quality of the sugarbeet crop.

According to W. E. McKeen (1949), *Aphanomyces cochlioides* is limited to the intercellular spaces of the cortical tissue of the root and hypocotyls before the cortex is sloughed off. Mycelium does not penetrate into the cotyledons of infected plants.

The early history of knowledge of the blackroot disease causal agent itself reflects some disarray in understanding. "Wurzelbrand" of sugarbeets in Germany was described by Peters (1906, 1911), and the cause attributed to *Aphanomyces laevis*. He recognized, however, that other organisms invading plants of *Beta vulgaris* induced similar symptoms in some cases. More than four decades later György (1957) still identified one of the beet root rot fungi as *A. laevis*. In any case, in 1912, Jensen isolated from soil a fungus which he identified as *A. laevis*, and said it was the cause of Wurzelbrand. Later, Edson (1915a) reported isolating an *Aphanomyces* (from Germany) identified by Peters as the same as the *A. laevis* which he found in roots of diseased sugar beets. After comparing the German specimen with isolates from Wisconsin and Illinois, Edson (1915b) concluded they were not the same, and named his American specimens *Rheosporangium aphanidermatum*. In 1929, Drechsler described *Aphanomyces cochlioides* propagated from diseased sugarbeets in Michigan, and concluded that the specimens could not be identified as *A. laevis*, yet probably represented the *Aphanomyces* which Peters had isolated. Curiously, Drechsler (1929:310) wrote that with the description of Edson's *R. aphanidermatum* as the parasite of beet roots, the references to *Aphanomyces* species associated with beet diseases in the U.S. would "...seem to come to an end." Contrary to Warren's statement (1948:884) that the species described by Edson (1915b) and later assigned to *Pythium* "...is now known as *Aphanomyces cochlioides*...", Drechsler nowhere in his account (1929) included such a decision. In any event, whether or not Peters was dealing with *A. laevis* as the blackroot disease organism or *A. cochlioides* cannot be determined; Edson's species has been put to rest properly in *Pythium*. Arrhenius (1923) published a lengthy account on some physiological aspects of root rot of sugar beets and although he mentioned *A. laevis* as one of the causes, this organism was not singled out in his treatment.

Although Schmidt (1934) concluded that a *Phoma* was the cause of blackroot of sugar beets and *Aphanomyces cochlioides* was but a secondary invader, and Esmarch (1942) reported this water mold as only one of three causes of the disease, others (Afanasiev, 1948a, b; Warren, 1948; Schäufele and Beiss, 1973; Drechsler, 1929) demonstrated that this *Aphanomyces* was a primary agent. Gonzalez (1975), studying "caida," a malady of sugar beets in Chile, noted that *A. cochlioides* usually occurred in association with other pathogens. In only one case in 100, he stated, was this species the sole causal agent. *Aphanomyces cochlioides* evidently can cause decay of sugar beets in storage as well as invade living plants (Cormack and Moffatt, 1961). According to Busse and Ulrich (1908), the fungus causing blackroot (identified then as *A. laevis*) does not attack beet seeds, and therefore is not a seed borne organism (*A. cochlioides*; Coons *et al.*, 1946). Winner (1966b) found that if mycelium of the causal organism was put into nonsterile soil, growth was suppressed. He concluded from this observation that hyphae had little function in the initiation of the disease.

Blackroot is widespread, and there are a number of publications simply reporting its occurrence, among them the following: Byford (1972, 1975, England), Campbell (1939, Puget Sound, Washington). Comerford and Mangan (1964, Ireland);

W. C. Moore (1959, England, Scotland), Murphy (1927, Ireland), Pidoplichko (1970, Russia), Steeger (1965, Chile) and Terényi (1928-29, Hungary). The disease also is known in West and East Germany (Beiss and Schäufele, 1973; Klemm *et al.*, 1957; Schäufele and Beiss, 1973; Schäufele and Winner, 1972; Winner, 1966a-c; Winner and Schäufele, 1968), Japan (Yokosawa and Kuninaga, 1977; Yokosawa and Ui, 1969), and Canada (Coulombe, 1969a, b, 1975; W. E. McKeen, 1949). Three general accounts of the disease are prominent: Hull (1976), Papavizas and Ayers (1974) and Schneider (1976).

FACTORS INFLUENCING DISEASE DEVELOPMENT

Soil Temperature: -- According to Winner (1962), warm soil temperature, possibly by a direct effect on the beet plants themselves, could be expected to support an increase in the severity of blackroot. Warm weather accompanied by heavy rainfall, Steudel (1969) maintained, also amplified damage from sublethal levels of *Aphanomyces* "type *cochlioides*". However, very little is known of the specific temperatures that influence disease development. Typical host damage by three isolates tested by Downie (1942) occurred when the soil temperature was in the range of 28-32 °C, and there were no lesions or symptoms developed in inoculated plants if the temperature was less than 25 °C. The incidence of blackroot rose, Warren (1948) reported, as the temperature increased, with greater damage occurring at 24°, 28°, and 32 °C than at 18° or 20 °C.

Soil Reaction: -- Reports on the effect of pH on blackroot development are few. In England, the disease occurs most frequently in beets planted in acid soils (Byford and Stamps, 1975). The disease intensity index of experimental plants, Fink and Buchholtz (1954) found, bore no correlation to soil pH.

Soil Moisture: -- There appears to be universal agreement among pathologists who have investigated blackroot of sugar beets that high moisture levels tend to elevate the disease incidence. Thus, disease damage and prevalence is high in plants grown in heavy soils, in undrained ones, and when there is prolonged wet weather (Busse *et al.*, 1911; Melhus *et al.*, 1939; Murphy, 1927; Steudel, 1969). A relationship between moisture and temperature and percentage of beet seedling infection has been demonstrated by W. E. McKeen (1949). When the soil moisture was at 20%, inoculated seedlings were not infected if incubated at temperatures from 5-25 °C; at 30% soil moisture, infection occurred in seedlings incubated at 17 °C and above. In wet soil (43% moisture level), inoculated seedlings developed blackroot at all temperatures tested above 5 °C (9, 13, 17, 21, 25 °C).

Soil Fertility: -- Downie (1942) concluded from field and greenhouse tests that provided a field had not been cropped frequently in beets and there was a low disease incidence, a high phosphate level reduced the incidence of blackroot caused by *Aphanomyces cochlioides*.

The results of Warren's (1948) study provide the same conclusion as does some experimental work by Winner (1966a, b). Coons (1953b), Coons *et al.* (1946), and Winner and Schäufele (1968) were of the opinion that a high level of soil fertility suppressed blackroot disease; Hildebrand and Koch (1943) concluded likewise, although they did not mention the fungal agent by name.

It cannot be said that the level of fertilizer in soil consistently influences the incidence of blackroot. At the end of a five-year period of a crop rotation experiment in Michigan, Schneider and Robertson (1975) reported that several rates of fertilization were ineffectual in modifying blackroot severity or incidence. Byford (1975), similarly, found no relationship between fertilizer use and blackroot incidence for beets in English soils.

In Japan, Yokosama and Ui (1969) experimented with soil type as a factor influencing the development of blackroot (as measured by estimates of severity and expression of symptoms). They used two soils, clay loam or loam enriched with humus derived from volcanic ash, and a loam-clay mix modified with alluvial soil. In every instance, plants grown in soil with volcanic ash were more severely infected by *Aphanomyces cochlioides*, and the disease symptoms appeared sooner than in plants propagated in soils not so amended. Water extracts of the soil with ash also appeared to raise the disease severity index when compared with diseased plants watered with extract from alluvial soils.

Yokosawa and Kuninaga (1977) described some effects of soil on viability and infectivity of the spores of *Aphanomyces cochlioides*. Spores remained infective in nonsterile soil for 20-30 days, but, of course, many were lysed, and motility was lost usually within one day. They also found that as few as 50 spores g⁻¹ of soil was sufficient to cause infection in susceptible beet plants.

RESISTANCE AND BREEDING PRACTICES

In general, the literature on the development of resistant cultivars of *Beta vulgaris* indicates that progress has been achieved (Schneider, 1964), but strictly immune varieties have not been developed. With greenhouse-grown plants, Henderson and Bochstahler (1946) analyzed the blackroot resistance capacity of 216 progeny from five parental strains showing various degrees of resistance. When one or both parents were to some degree resistant, the progeny also gave a better yield than when neither parent was resistant. Doxtator and Downie (1948), Doxtator *et al.* (1950), Doxtator and Finkner (1954), Downie *et al.* (1952), Downie, Doxtator *et al.* (1952), Gaskill *et al.* (1948), Bochstahler *et al.* (1950), and Bochstahler and Reese (1948) all reported improved resistance to blackroot in sugar beet lines resulting from breeding practices. According to Bochstahler *et al.* (1950), resistance is a genotypically dominant factor; Coe and Schneider (1966) obtained increased degrees of resistance to blackroot in certain strains by means of selections over several generations and concluded that tolerance was not inherited by a single gene factor.

No breeding lines tested by Afanasiev (1956), Afanasiev and Morris (1954), and Afanasiev and Sharp (1961) consistently exhibited a high degree of resistance to *Aphanomyces cochlioides*, and there were noticeable differences in degree of disease incidence in breeding lines grown in greenhouse versus field testing sites. By contrast, Coons (1953a), and Coons *et al.* (1952, 1955) demonstrated that through mass breeding and selection programs, cultivars could be established combining resistance to leaf spot disease (*Cercospora beticola*) and blackroot. None of the lines tested by them was immune to the disease caused by the *Aphanomyces*, but reduction in visible damage was one bit of evidence that breeding had to some extent succeeded.

To what extent are foreign introductions resistant to (or tolerant of) the "native" North American strains of *Aphanomyces cochlioides*? Plants from 217 seed entries were inoculated by Schneider and Gaskill (1962) with suspensions of spores of *A. cochlioides* and subsequently scaled for disease severity in comparison with that exhibited by an inoculated, selected U. S. variety. They found 93% of the foreign introductions to be more susceptible to *A. cochlioides* than the check variety. Annual types of beet cultivars were generally less susceptible than the biennial ones, and mangels were the most susceptible of all the varieties tested.

There are but two published reports that treat specific aspects of the reaction of beet cultivars to *Aphanomyces cochlioides*. Stomatal and cuticular resistance in inoculated and uninoculated beet cultivars was determined by Safir and Schneider (1976). Seedlings inoculated with the fungus had a lower transpiration rate and a higher diffusive resistance than did the uninoculated ones. The increased diffusive resistance in the inoculated plants was initiated by a curtailed water supply to the leaves. These investigators suggested that this parameter -- water flow movement -- might be used in estimating beet blackroot severity.

The nature of resistance and susceptibility of sugar beets to *Aphanomyces cochlioides* was studied histologically and histochemically by Herr (1975). In both resistant and susceptible varieties of the host the mycelium was largely intercellular, did not grow in advance of the margin of the lesions, and only in cases of very severe infection penetrated into the vascular cylinder. In the stelar region adjacent to lesions, peroxidase and (at times) phenolase activity was high, and phenols and quinones accumulated in these same stelar areas. Cell wall constituents in diseased plants were not much different, Herr reported, from those in healthy plants. He suggested that the stele might play a prominent part in the interaction between the hyphae of the invading fungus and the host tissue.

HOST RANGE

Aphanomyces cochlioides is by no means as broadly adaptable to various hosts as its relative, *A. euteiches*, seems to be. However, just as in the case of the latter, the host range for *A. cochlioides* has in part been determined by analysis of pathogenicity in sterile soil, and in part by growing possible hosts in naturally infested soil. There is,

then, an artificiality to the host range, and it must not be interpreted as necessarily indicative of the hosts under more natural field conditions.

The largest single exploration of the host range of *Aphanomyces cochlioides* was that conducted by Schneider (1965). He tested in the laboratory and greenhouse 98 vascular plant species in 40 families finding 28 species that had not previously been reported as hosts for this fungus. Earlier, Buchholtz (1944b), Downie (1942), W. E. McKeen (1949), Melhus *et al.* (1939), and Schmitthenner (1964) also had studied the host range of the causal agent. The results from the latter's work are particularly revealing. Schmitthenner tested the susceptibility of seedling host plants in greenhouse experiments (in flasks), and found that *A. cochlioides* invaded two crucifers also known to be hosts for *A. euteiches*: *Brassica oleracea* var. *capitata*, and *Raphanus sativus*. Other hosts common to these two pathogens are cited in Table 45. A listing of additional species susceptible to *A. cochlioides* may be found in the review by Papavizas and Ayers (1974: table 2, pp. 19, 20). Interestingly enough from a taxonomic viewpoint, both *A. cochlioides* and *A. euteiches* have been reported to occur in *Beta vulgaris* (L. E. Carlson, 1965, excised root tip method; Buchholtz, 1944a; Drechsler, 1929; Downie, 1942; W. E. McKeen, 1949; Schneider, 1965).

INTERACTIONS

As has been demonstrated to occur with *Aphanomyces euteiches* (Chapter 27) *A. cochlioides* also interacts *in vitro* and *in vivo* with other organisms. According to Drechsler (1943a), hyphae of *A. cochlioides* in culture growing in opposition to *Pythium myriotylum* Drechsler entwined loosely about the pythiaceus filaments, and on a modest scale even invaded them. On the other hand, little injury occurred to the hyphae of *P. acanthicum* Drechsler, *P. oligandrum* Drechsler, and *P. periplocum* Drechsler when grown with the beet blackroot fungus. When *A. cochlioides* was inoculated into beets propagated in nonsterile soil, Lyda (1958) reported, species of *Alternaria*, *Penicillium*, and *Pythium* evidently were antagonistic to it because the disease incidence was lowered. No such reduction occurred if sterile soil was used. Bissonette (1964) found that *Erwinia caratovora* (Jones) Bergey, Harrison, Freed, Hammer and Huntoon often was associated with *A. cochlioides*. Coulombe's (1975) experimental work showing that table beets are more susceptible to the agents of blackroot than sugar beets also indicated that certain angiospermous weed species influenced beet growth as reflected in disease incidence.

The possibility that the nematode, *Heterodera schachtii*, was implicated in sugar beet blackroot was explored by E. D. Whitney and Doney (1970, 1973). While neither the nematode nor the *Aphanomyces* alone could kill beet seedlings, there was a noticeable additive effect where the two species were used in combination at high inoculum levels. However, a critical discovery was made: the sum of the effects on beet yield of the two organisms alone was greater than that expressed by them in combination. There is evidence (Whitney and Doney, 1973) that large populations of *H. schachtii* predispose sugar beets to infection by *A. cochlioides*. There is an increased loss

of sugar beet plants infested with aphids or infected by virus if they were at the same time being invaded by the blackroot fungus (Steudel, 1972).

CONTROL AND SUPPRESSION

Several direct methods of control of blackroot have been attempted, notably seed treatment (the fungus is not seed-borne) and application of fungicides. Chiefly, however, the agronomic practice of crop rotation has been experimented with most widely. Some selected publications treating control of the disease are listed in the paragraphs to follow.

Application of Chemicals: -- Seed treatment, and the application of such fungicides as Dexton, Captan, Arasan, and pentachloronitrobenzene (PCNB), have been used to suppress blackroot, and generally with some positive reduction in severity and a corresponding increase in yield. Effectiveness of the applied chemical is, of course, related to a number of factors -- conditions at the time of application, method of application, and concentration of the compound, among others. Publications by the following may be consulted: Afanasiev (1948b, 1962), Buchholtz (1944a), Byford and Prince (1976), Coons (1953b), Coons *et al.* (1946), Coulombe (1969a, b), Downie (1942), Gonzalez (1975), A. A. Hildebrand *et al.* (1949, 1951), Hills (1962), Hills and Leach (1952), Kanzawa (1968), Lewis and Papavizas (1971b), Lyons *et al.* (1954), W. E. McKeen (1949), Schäufele and Winner (1972), Steudel (1968, 1969), Sugawara (1966), Tverskoï (1954), Winner (1962; 1966a, c), and Winner and Schäufele (1968). Other chemicals have also been tested for possible usefulness in disease control as, for example, lime (Terényi, 1928,-29), penicillin and streptomycin [for seed treatment (W. E. McKeen, 1949)], and isomers of methionine and various homologues (Winner, 1966c).

Many reports of fungicide testing are excluded from the forgoing illustrative listing, since they are strictly of an applied nature, and treat results in terms of economic impact. The results of single season fungicide tests (Schneider and Potter, 1969) are arbitrarily ignored. It should be emphasized that the use of fungicides and seed treatment chemicals is not universally accepted as a propitious means of controlling the disease (Buchholtz, 1944a; Byford, 1972).

Organic Residues and Inorganic Amendments: -- The experimental use of plant residues (and fertilizers) in the suppression of blackroot of sugar beets has not been as extensive as their use in the preventive medicine, as it were, applied to pea root rot. Results are somewhat varied among the few studies that have been published, but there is an indication that well-balanced fertilizer application and certain plant residues (tissues of crucifers, notably tend to reduce yield loss due to blackroot (Bellingham, 1949; Lewis and Papavizas, 1971b; Lyda, 1958; MacWithe, 1967).

Agronomic Practices (Crop Rotation): -- Experimental evidence (largely from laboratory and greenhouse work) demonstrates that certain crops preceding sugar beets

have a profound effect on the severity of blackroot when sugar beets again are planted in the field. There is equally convincing evidence that not all crop plants have an effect. Some examples will illustrate the nature of cropping sequences.

Bellingham (1949) determined (greenhouse testing using fumigated soils) that the amount of blackroot was decreased if sugar beets were planted after alfalfa or corn had grown in the soil, but was increased if sugar beets followed plantings of potatoes, beans, beets, or corn in the cropping sequence. Quite to the contrary, Coons *et al.* (1946), found that legumes (such as alfalfa and clover) grown on land in years prior to sugar beets actually favored development of the disease. Coons *et al.* (1946) and Deems and Young (1956) reported that a rotation in which corn crops preceded beets resulted in a greater yield of sugar beets and a suppression of the disease. None of the rotational crops used in studies by Bissonnette (1964) had a beneficial effect by reducing blackroot disease incidence.

Perhaps it may be fairly said that the alleged efficiency of cropping sequences is not understood thoroughly or explored. For example, Schneider (1965) demonstrated that legumes were not hosts for *Aphanomyces cochlioides*, yet there is evidence that such crops in a rotation sequence ahead of sugar beets seem to favor a high blackroot disease incidence. Additional publications which may be consulted on cropping sequences are those by Buchholtz (1944b), Byford (1975), Coons (1953b), Downie (1942), Fink and Buchholtz (1954), Lyda (1958), Melhus *et al.* (1939), Schneider and Robertson (1975), and Steudel (1968, 1972).

EXPERIMENTAL METHODS

Various investigators working with *Aphanomyces cochlioides* have developed specific experimental techniques to yield information of a particular nature about blackroot.

In the study of disease-causing fungi where inoculation procedures are anticipated, some knowledge of inoculum load is necessary. To differentiate between a susceptible sugar beet variety and a resistant one, Schneider (1954) found it necessary to inoculate test plants with 1×10^5 - 2×10^5 spores of *Aphanomyces cochlioides* mL⁻¹ of inoculum carrier. He concluded that in general the degree of resistance expressed by a given cultivar of sugar beets grown in the greenhouse was the same as that which could be expected in the field. MacWithey (1961) attempted to determine, *in vitro*, the number of spores necessary for infection. He tested inoculum loads as few as 20 spores to those containing as many as 2×10^5 spores mL⁻¹ of inoculum carrier, finding that seedlings could be infected at any of the spore concentrations used, but at 2000 spores mL⁻¹ and above, the percentage of infection reached 64-80%. In addition, MacWithey studied the influence of inoculum exposure duration on subsequent incidence of infection. *Aphanomyces cochlioides* invaded susceptible beet cultivars when these were exposed to spore inoculum for periods ranging from 2-24 hours. The highest percentage of infected seedlings occurred at exposure times of 16-24 hours.

Initiation of infection of sugar beets, Schneider and Yoder (1973) determined, could be attained by inoculating susceptible plants with oospores of *Aphanomyces cochlioides*. They calculated the amount of homogenate (mycelium plus oospores) needed to provide a given number of oospores, based upon the yield of these cells in culture. The resulting severity of blackroot in sugar beet seedlings following inoculation with oospores (specifically the product of oospore germination) of *Aphanomyces cochlioides* was recorded from a series of planting tests conducted by Schneider (1978). Inoculum placed below the soil surface resulted in a higher disease severity index (see Chapter 27 for definition) than did inoculum applied only on the soil surface. If the germinable oospores were mixed with the potting soil at the time the beet seeds were planted the resulting DSI was higher than that obtained if inoculum was spread in the soil six days after the seeds were planted. Oospores generally were effective as inoculating agents, Schneider noted, after more than one year in storage at 4° or -9 °C. With prolonged storage, however, the oospores became less effective as a source of inoculum.

A degree of uniformity in inoculation must be attained if comparative studies on disease resistance, for example, are to yield meaningful data. Schneider (1959) experimented with three media as inoculum carriers for *Aphanomyces cochlioides*: steamed soil, oat grain, and a sorghum-grain preparation. These three carriers proved to be suitable for field inoculation provided they were drilled into the soil. Herr (1974) inserted a soft paraffin barrier into containers containing beet seedlings and could, by manipulating the paraffin, insure that the plants were inoculated by *A. cochlioides* at the hypocotyl or epicotyl alone.

Fink and Buchholtz (1954) concluded that it was possible to use disease severity indices calculated from the results of greenhouse inoculations (with the causal agent) of sugar beets as a reliable guide in selecting the fields for planting. In a two-year sequence of testing, they found correlation in three of 19 and two of 24 fields, respectively.

To explore the possibility that green plant residues used as soil amendments were capable of supporting colonization and sporulation by *Aphanomyces cochlioides* in the soil -- and thereby likely would contribute to a high incidence of blackroot -- MacWithey (1967) measured the sporulation capacity of the fungus on various residues. The resulting data were clear: the level of blackroot disease could be a measure of the capacity of the crop residue to support growth and reproduction of the pathogen. As to the collection of *A. cochlioides* from soil, MacWithey was able to recover it from comminuted organic debris in soil, but not by using an immersion tube or dilution plate technique.

Foliage vigor and relative quantity of plants in field-grown sugar beets were rated by Schneider and Safir (1975), and the resulting data compared with ratings determined by a color scale index of infrared photographs of those same fields of beets. Calculated coefficients of correlation between root yield and infrared index were statistically significant, as were those between photo color rating and ground-surveyed

determinations. Infrared photography thus provided a rapid and convenient method of plot evaluation.

As has been pointed out with reference to *Aphanomyces euteiches* (Chapter 27), some degree of correlation exists between disease severity indexing in greenhouse trials and the expected prevalence of pea root rot in the field. It appears from the work of Bartels and Winner (1971), however, that forecasting of blackroot severity from results obtained with infected seedlings of plants grown in pots cannot be done without considerable qualification. They suggested that pot trial results have only a limited usefulness in formulating predictions of field losses.

RADISH BLACKROOT (*Aphanomyces raphani*)

SYMPTOMATOLOGY AND THE PATHOGEN

Aphanomyces raphani, described by Kendrick in 1927, is not the same as Edson's *Rhasporangium aphanidermatus* (1915b), also isolated from *Raphanus sativus*; the latter is a *Pythium*. The *Aphanomyces* invades the root tissue of radish (the edible portion is largely hypocotyl, not root, and, according to Kendrick, is not penetrated) possibly through fissures at the sites where secondary roots exit (Böning, 1932-33; Wenham, 1960) or at wound sites.

Discoloration of the invaded, subepidermal tissue is an early symptom of radish blackroot, and is followed by a distinct blackening. Discoloration proceeds in a transverse fashion (Böning, 1932-33), and thus accounts for the banded appearance of infected roots. Viewed from outside the root, the blackened interior tissues appear grayish-black (Herold, 1964). Softening of tissues invaded by *Aphanomyces raphani* also accompanies the development of infection, but this is alleged to be the result of penetration by bacteria (Böning, 1932-33; Kendrick, 1927). Following bacterial invasion, root tissues show signs of disintegration. In advanced stages of the disease, the symptoms are expressions of stunting, blackened lesions, girdling, and root deformity (Wenham, 1960; Weber, 1932).

As the disease progresses, *Aphanomyces raphani* invades the hypocotyl, cotyledons, petioles, and leaf blades of seedlings. Kendrick (1927) stated, however, that it was not known how the organism entered these other tissues. Oospores of *A. raphani* occur *in vivo* only in the outer, disintegrating tissue, and sporangia evidently are not formed (Kendrick, 1927) in the infected roots. Ghafoor (1964) reported, however, that oospores of the fungus were produced abundantly in invaded secondary roots, and were to be found also in cortical tissue free of symptoms. Herold (1964) found oogonia and antheridia of *A. raphani* in the border of infected regions. The fungus evidently is not seed-borne (Kendrick, 1927; Weber, 1932).

According to Yokosawa *et al.* (1972, 1974) *Aphanomyces raphani* causes damping off of cabbage. The spores of the fungus are attached to and accumulate (tactic response, in culture) on the roots and hypocotyls of both host and "nonhost" plants. In cases where the spores become attached to the hypocotyl (and subsequently germinated

there) the resulting infection was severe (Yokosawa *et al.*, 1972). Conversely, if the germ tubes of the spores penetrated only the cabbage root tissue, the disease incidence was low. Thus, it appears that *A. raphani* largely is an invader of roots in radish, but of the hypocotyl in cabbage.

With some reservation, Pavgi and Singh (1969) associated *Aphanomyces raphani* with a root disease of low vigor transplants of *Brassica oleraceae* var. *botrytis* (cauliflower) in poorly aerated, waterlogged clay soils. Later, Singh and Pavgi (1977a, b) described the pathogen on cauliflower as a new species (*see*, in taxonomic account, *A. raphani*).

FACTORS INFLUENCING DISEASE DEVELOPMENT

Very little has been done to determine the conditions under which blackroot of radish develops. Böning (1932-33) stated that high summer temperatures favored the disease, as did excessive use of organic fertilizer and failure to practice crop rotation. If the mineral salt level of soil in which radishes were grown was increased (experimentally), Herold (1952) found that the infection level in plantings was raised correspondingly. Radish blackroot was more severe in seedlings grown in sandy soil than in composted loam. The addition of boron to a deficient soil in which radishes were infected severely did not reduce the prevalence of the disease, according to Hosford (1961).

RESISTANCE

Eighteen varieties of radish were greenhouse tested by Kendrick (1927) for resistance to *Aphanomyces raphani*, 23 were grown in field soil known to be infested by the fungus, and 14 were propagated in soil in which radishes had been grown for three successive years. None of the radish types was immune, although one cultivar was not infected during either the greenhouse tests or during those experiments using infested soil. Plants of this variety became diseased, however, when grown in the soil cropped successively to radishes. Humaydan *et al.* (1976) tested for resistance 30 cultivars from six breeding lines. There was wide variation in the degrees of susceptibility expressed but some selections that had been screened for resistance in the greenhouse testing maintained that resistance when planted in the field.

CONTROL

In the original account of blackroot of radish, Kendrick (1927) stated that there was no control for the disease, but losses could be reduced by using red globe varieties of radish rather than the long, white ones which usually were more severely damaged by the causal agent. Weber (1932) recommended that control practices include crop rotation and the use of resistant varieties (or planting susceptible ones only in soil not previously cropped to radish), and Wenland (1976) essentially echoed these views in his review of the disease problem. Planting radishes in noninfested ("new") soil rich in

humus, and in areas of low moisture and low temperature, Herold (1964) concluded, would reduce the chances of the host becoming infected. Fields under radish cultivation for several seasons evidently become so heavily infested with the fungus that the land is then unsuited for further radish production (Humaydan *et al.*, 1976).

As to the use of chemical therapy in the control of the disease caused by *Aphanomyces raphani*, the information is scanty indeed. Böning (1935-36) was able to control blackroot by mixing calcium cyanamide, ammonium sulfate, and formalin into the soil before planting the host seeds. This rather harsh treatment also retarded growth of the radish. Lime and Ceresan mix gave some control (these compounds did not eliminate the disease from fields), but fertilization with urea was not effective. Chloropicrin (Hosford, 1961) reduces the incidence of radish blackroot initially, but this suppressive effect does not persist.

HOST RANGE

Aphanomyces raphani appears to be limited to occurrence in cruciferous plants (Humaydan and Williams, 1975; Kendrick, 1927; Ogoshi, Saki, and Yokosawa, 1972; Ogoshi, Yokosawa, and Saki, 1972; Yokosawa *et al.* 1972). Ghafoor (1964), for example, reported that the causal agent did not infect (artificial inoculation) species of Chenopodiaceae, Compositae, Cucurbitaceae, Leguminosae, Malvaceae, Solanaceae, or Umbelliferae. Table 46 records the plant species known to harbor *A. raphani*; some of these hosts were of course determined (Ghafoor, 1964) strictly through artificial inoculations. All crucifers tested in the field by Humaydan and Williams (1975) subsequently were found to contain oospores of the fungus in the root tissues.

MISCELLANEOUS PARASITES AND PATHOGENS

APHANOMYCES CLADOGAMUS

Drechsler first reported *Aphanomyces cladogamus* in 1927, as the cause of injury to roots of *Lycopersicon esculentum* Mill., but did not formally describe the species until 1929. The fungus evidently is only a very weak parasite in the plants it is alleged to invade.

Symptoms of root damage caused by *Aphanomyces cladogamus* have been described for tomato, *Viola* spp., and *Capsicum frutescens*, L. In tomato rootlets, the fungus induces at first a discoloration then subsequently kills the invaded tissue (Drechsler, 1929). The *Aphanomyces*-invaded cortex of pansy roots is softened when first penetrated but in time the tissue becomes discolored, and subsequently disintegrates as the infection progresses (Drechsler, 1954c). Wilting of invaded plants may be a symptom even when the roots show no evidence of disease. In cases of severe infection by *A. cladogamus* the root and stem cortex are damaged, and oogonia are found within the vascular cylinder.

Seedlings of pepper attacked by *Aphanomyces cladogamus* show blackening and necrosis of the hypocotyl region and the base of the cotyledons. Initially, the infected area is soft, and in time the invaded subtending cortical tissues also become necrotic. The fungus causes in peppers both pre- and postemergence damping off (C. D. McKeen, 1952). Once the first leaves have developed on the seedlings, however, the plants evidently are no longer susceptible to invasion.

Drechsler (1929) succeeded by artificial inoculation in inducing *Aphanomyces cladogamus* to attack roots of *Beta vulgaris* L. In this instance, too, the fungus proved to be a weak parasite. Several other plants are reported as hosts for the *Aphanomyces* (Drechsler, 1954a, c; C. D. McKeen, 1952): *Allium cepa* L., *Linum usitatissimum* L., *Raphanus sativus* L., *Solanum melongena* L., and *Spinacia oleracea* L. If Scott (1961) is correct in assigning the *Aphanomyces* sp. reported by Mix (1945a) to *A. cladogamus*, then lettuce (*Lactuca* spp.) must be added to the host list.

The host range of *Aphanomyces cladogamus* is not far removed from that of *A. euteiches*. The *Aphanomyces* (thought to be *A. euteiches*) isolated by Buisman (1927) from roots of *Viola* species possibly was *A. cladogamus*. The same may be said for *A. euteiches* P. F. 2 collected by Muers (1928), although it should be noted that *Viola* species are listed by Papavizas and Ayres (1974) as hosts for this pea root rot fungus. The morphological differences between the two species -- ones of decidedly little dimension -- coupled with the obvious host range overlap, is strong evidence indeed that they are but one species (see systematics section).

Drechsler (1943b, 1946) studied *in vitro* antagonistic relationships between *Aphanomyces cladogamus* and species of *Pythium*. Hyphae of this *Aphanomyces* were shown to envelop and injure (with or without penetration) the following species: *P. acanthicum* Drechsler, *P. anadrum* Drechsler, *P. myriotylum* Drechsler, *P. oligandrum* Drechsler, *P. ostracodes* Drechsler, *P. salpingophorum* Drechsler, and *P. vexans* de Bary.

APHANOMYCES IN OATS (*Avena sativa* L.)

In 1931(b), Brandenburg reported the occurrence of *Aphanomyces* sp. from the roots of oats grown in the Netherlands and Germany. When the associated fungus was isolated and then inoculated back into the supposed host, some tissues showed signs of disease, but others did not. Roots in which the fungus became established exhibited symptoms of discoloration in the stele, and oospores were present in the cortex.

Whether Brandenburg's isolate was the same as Drechsler's *Aphanomyces camptostylus*, also isolated from roots of *Avena sativa*, cannot be said with certainty. In any case, Drechsler (1929) only had cultures of the fungus, and symptomatology at the time he isolated the species evidently was not recorded. Drechsler suggested that oats in poorly drained soils became chlorotic, but he did not directly implicate *A. camptostylus*. This species damaging to oats is destructive to artificially inoculated beet seedlings also, according to Drechsler (1929).

APHANOMYCES IN ALFALFA (*Medicago sativa* L.)

An unidentified species of *Aphanomyces* was reported by Schmitthenner (1964) to be moderately virulent in roots of alfalfa, and less so in roots of *Trifolium pratense* L. Invaded tissue was necrotic but not disintegrated. The fungus would attack only alfalfa and red clover when artificially inoculated onto roots of these species. Morphologically, the *Aphanomyces* was quite similar to *A. euteiches*.

APHANOMYCES IN CAULIFLOWER (*Brassica oleracea* var. *botrytis* L.).

In 1969, Pavgi and Singh reported that *Aphanomyces raphani* had been isolated from cauliflower. Subsequently, however, Singh and Pavgi (1977) identified the pathogen as a new species, *A. brassicae* (see systematics section).

A soft, water-soaked condition is an early symptom of the *Aphanomyces* root rot of cauliflower, but eventually the invaded cortical tissues become discolored. The vascular cylinder of the host remains intact even after the cortex has sloughed off, a condition identical to that in the case of *A. euteiches* infection of peas (Chapter 27). Growth of the infected plants is retarded by *A. brassicae* (= *raphani*), the lower leaves wilt, and secondary invaders enter, hence there is no recovery (Singh and Pavgi, 1977a). Disease severity is highest in plants in moist soils and in clay soils.

Singh and Pavgi (1977b) artificially inoculated roots of *Brassica oleracea* var. *capitata* L. and *B. oleracea* var. *caulorapa* with *Aphanomyces brassicae*, and these plants succumbed to the disease. No infection developed, however, in inoculated plants of *B. campestris* L., *B. rapa* L., and *Raphanus sativus* L. Evidently the germ tubes from planonts of *A. brassicae* can penetrate susceptible root tissue (of cauliflower) at the epidermis in the region of elongation, and at the root hair surface. Singh and Pavgi (1977b) reported that the spores of the causal agent actually penetrated root hairs, but also in their paper contradicted this observation hence this point is unclear. Planonts of *A. brassicae* respond chemotactically first to the root's region of cell elongation, then to the root cap, and ultimately are attracted to the root hairs. An explanation for the latter unusual response is wanting.

APHANOMYCES EXOPARASITICUS

Described in 1926(a) by J. N. Couch, *Aphanomyces exoparasiticus* (isolated from hyphae of a *Pythium*) is considered to be a "hemi-saprophyte" capable of attacking a number of phycomycetous fungi. In two-member cultures, *A. exoparasiticus* reacts in various ways when growing with other fungi. In some cases, the host fungus is parasitized and inhibited, but the *Aphanomyces* is not retarded. This reaction occurs (J. N. Couch, 1926a) in cultures of *A. exoparasiticus* with *Achlya orion*, *A. megasperma*, *Dictyuchus monosporus*, *Pythiopsis cymosa*, *Thraustotheca clavata*, *T. primoachlya* (= *Achlya primoachlya*), and *Pythium* spp. Both the host fungus (the one parasitized by the *Aphanomyces*) and *A. exoparasiticus* itself can be inhibited in this "mutual" relationship; all species of *Mucor* and *Rhizopus* tested also reacted in this fashion. Mutual parasitism

and mutual inhibition occurred with all test species of *Aphanomyces* and *A. exoparasiticus*, Couch found. In cultures of *A. exoparasiticus*, and *Syncephalis* sp., the mucoraceous host was at first parasitized, but subsequently overgrew the *Aphanomyces*. When *A. exoparasiticus* was grown in culture with species of *Sordaria*, *Aspergillus*, and *Penicillium*, there was mutual inhibition but no evidence of parasitism. However, when the *Aphanomyces* was in contact with *Schizophyllum commune* Fr., the basidiomycete was inhibited and its mycelium damaged. At room temperature, *A. exoparasiticus* overgrew and parasitized *Allomyces arbuscula* E. J. Butler, but, at 34 °C, the *Aphanomyces* was overgrown, and there was no parasitic response.

SPECIES OF PLECTOSPIRA

Plectospora marinara (Drechsler, 1927) was originally found in the roots of potted tomato seedlings in a greenhouse. It was associated with a brownish discoloration of injured roots, and occasionally with dead root tops. Of a second species, *P. gemmifera*, which he isolated from a diseased root of *Saccharum officinarum* L., Drechsler (1929:350) wrote "...the fungus presumably had been present in a parasitic relationship." Neither species has again been reported. *Plectospora myriandra* was demonstrated (Drechsler, 1946) to be antagonistic to *Pythium anandrum*, *P. undulatum* Petersen, and *P. vexans*.

KIKUYU YELLOWS

This is a disease of *Pennisetum clandestinum* (a pasture grass in New South Wales), recognized by stunting and chlorosis of the emergent portions of infected plants, and discolored and partly decomposed roots (Wong, 1975). The pathogen, probably a saprolegnian (although we have not been able to confirm this from preserved specimens at hand), induced symptoms of the disease in artificially inoculated seedlings, and was recovered (Wong, 1975) from those plants subsequently.

SAPROLEGNIAN DISEASE OF MUSHROOMS AND GRASS

In 1884, W. G. Smith described *Saprolegnia mucophaga* as a parasite of the gills of *Agaricus campestris* Fr. The watermold was alleged to have spherical sporangia and pyriform oogonia, but was illustrated as possessing septate hyphae! It seems clear that Smith's considerable ability as an artist was not matched by a degree of competence with the fungi.

According to his paper published in 1884, W. G. Smith described another watermold, *Saprolegnia philomukes*, in 1882. The one illustration of a fungus at the edge of a grass leaf (W. G. Smith, 1882: fig. 58, in part) is by no stretch of the imagination a watermold. (See *Saprolegnia*, excluded taxa, in the systematic account.)

WATERMOLDS ASSOCIATED WITH RICE (*Oryza sativa* L.)

Various species of Saprolegniaceae have been implicated in decay or disease of rice seedlings and seeds. In 1912, Sawada reported that *Achlya proliferata* (with which he included *Dictyuchus magnusii*) was the chief cause of rice seedling decay in Formosa (Taiwan). Additionally, he isolated from diseased plants an unidentified *Saprolegnia*, and two "species" of *Dictyuchus*. Hemmi and Abe (1928) also implicated *A. proliferata* in seed and seedling rot of rice (as did Takahashi, 1918, and Abe, 1928), but believed it was not excessively damaging to the host. From the results of some experimental seedling culture work using medium containing staling products from growth of *A. proliferata*, Hemmi and Abe demonstrated that the medium alone could reduce the growth of rice seedlings. They concluded that *A. proliferata* produced some toxic substance detrimental to *Oryza sativa*.

Corbetta (1956) found some saprolegniaceous fungi (which he did not identify) associated with a rot of weakened rice seedlings. These fungi also appeared on seed samples having low germinability. According to Corbetta, the "disease" was most prevalent during cool weather. There is nothing in his account, however, to suggest that the fungi were active pathogens. As had Corbetta, Wei and his colleagues (1955) found water molds (*Achlya* species) associated with rice seedlings presumably weakened by low temperature or by reduced oxygen tensions.

Four of the water molds most frequently isolated from various substrates -- *Achlya americana*, *A. flagellata* var. *yezoensis* (= *debaryana*), *A. oryzae* (= *debaryana*), and *Dictyuchus sterile* (excluded name; see taxonomic account) -- were used by S. Ito and Nagai (1931) in an extensive series of inoculations of rice seeds (and seedlings) to determine pathogenicity. Both hulled and unhulled seeds were tested, with hulled ones showing the greatest degree of infestation by the water molds. Only the *Dictyuchus* was unable to infest the seeds. In addition, high percentages of seedling infection were obtained with *Saprolegnia anisospora*, *S. diclina*, *S. thureti* (= *ferax*), and *Isoachlya itoana* (= *S. itoana*).

Rice seedlings were grown by T. Ito (1943) in nutrient solutions and then inoculated with various species of water molds [*A. oryzae*, *A. racemosa*, *Aphanomyces helicoides* (= *laevis*), and *Saprolegnia mixta*, for example. Only rather low incidences of infection resulted. To some extent, the ability of some isolates to damage the seedlings substantially was related to the temperature of incubation (there is such considerable overlap among temperature ranges reported by Ito that the specific data on infection percentages do not really indicate any trends). Ito concluded that species of Saprolegniaceae were not as important as agents in rice seedling rots as were species of *Pythium*. Among pythiaceous fungi on infested rice grains S. Ito and Tokunaga (1933) found a scattering of saprolegniaceous hyphae, but did not isolate any of them or identify any fungi that developed from those filaments. Their report leaves no doubt that they considered species of *Pythium* to be the chief culprits in seed and seedling rot of rice.

Webster *et al.* (1970) obtained three cultures of *Achlya klebsiana* from diseased rice seedlings, and from rotted seeds, and then carried out a series of experiments on their pathogenicity. All three isolates were pathogenic to seedlings, and at both high (30 °C)

and low (20 °C) temperatures of incubation. However, once the seedlings were established vigorously, *A. klebsiana* (= *debaryana*) was ineffectual as a pathogen. According to Dogma (1975), *A. flagellata* (= *debaryana*), *A. proliferoides*, and *A. diffusa* -- in addition to *A. klebsiana* -- were pathogenic to viable rice seeds and seedlings in the Philippines. Ungerminated seeds, inoculated with secondary spores of these species, evidenced infection in three days; germinated seeds showed signs of invasion by the watermolds one day after inoculation. All isolates were infective at incubation temperatures of 15, 25, and 30 °C.

The study by Oelke *et al.* (1969) of seedling resistance demonstrated that rice seeds with a moisture level below 20% were much more resistant to invasion by *Achlya klebsiana* than were those with a higher moisture content. The former also germinated earlier than the latter (vigor tests), hence there appeared to be a relationship between seed moisture content and enhanced resistance to invasion by this watermold.

Among the plant diseases in Japan forecast by Salmon (1951) was seedling rot of rice. He listed *Achlya* spp. (and *Pythium* species) as the causal agents of rots of this angiosperm, and predicted that the incidence of seedling rot would be likely to rise following periods when the water temperature in seedling paddies was at 13 °C for several days.