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BLACK ROT OF CARROTS CAUSED BY ALTERNARIA RADICINA N. SP.[1]

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WITH PLATE XI AND TWO FIGURES IN THE TEXT

During the winter of 1918–19 dealers at the New York market frequently complained of heavy losses due to decay of carrots in transit and storage, particularly in the case of stock that had been held in storage on Long Island farms. For this reason, early in April a trip was made by one of the writers to farms in the vicinity of Peconic and Orient, New York, for the purpose of studying local storage conditions.

The farmers living in this section of Long Island usually store their carrots in one of three ways; either in narrow pits, in mounds above ground or in cellars. During the course of the investigation it was found that irrespective of the method of storage used, a few of the carrots in most lots examined were either partially or entirely destroyed by a characteristic black rot. Examination of stock stored on farms in Danvers and South Peabody, Massachusetts, brought to light the fact that this disease is prevalent in these communities also.

The trouble, as it occurs on the root, is characterized by a progressive softening and blackening of the tissues. Infection seems to occur frequently at the crown, as a result of which the black decay extends down the core of the carrot, as shown by the photograph in plate XI, A. Many cases have also been observed where the decay had originated at other points on the surface of the carrot. Observations made on the crop in storage in the several localities mentioned in this paper indicate that although the disease is by no means as serious as that caused by *Sclerotinia sp.*, it is, nevertheless, responsible for considerable loss each winter.

[1] The work discussed in this paper was done as a coöperative enterprise between the Office of Cotton, Truck, and Forage Crop Disease Investigations and the Bureau of Markets, United States Department of Agriculture, partly at Washington, D. C., and, through the courtesy of the Director, Dr. G. S. Gager, partly in the laboratories of the Brooklyn Botanic Garden, Brooklyn, N. Y.

On the above mentioned field trips, specimens were collected at Peconic, Southold, and Orient, New York, and at Danvers and Peabody, Massachusetts. The fungus was isolated from these specimens by transferring small pieces of infected tissue from a freshly exposed surface to hard potato agar tubes by means of sterilized instruments. Pure cultures of an undescribed fungus, which was later identified as a new species of Alternaria, were obtained from each carrot affected by the black rot. The fungus developed rapidly on potato agar, forming a dense, black mycelial growth over the surface, and after a few days produced conidiophores and conidia in great numbers.

INOCULATIONS OF CARROTS

In order to determine the relation of this organism to the decayed tissue from which it was isolated, a series of inoculations were made. Three dozen sound carrots were washed thoroughly with water and then subjected to surface sterilization. This consisted of washing the root quickly with 95 per cent alcohol, this treatment followed immediately by application of a mercuric chloride solution (1 to 1000). After the latter solution had been allowed to act for 5 minutes, the carrots were rinsed thoroughly in sterilized water. Three incisions were then made on each carrot by means of a flamed scalpel, and small masses of mycelium and spores taken from pure cultures of the fungus were inserted in the incisions on 28 specimens. The remaining 8 were not inoculated but were retained to serve as controls during the experiment. All of the carrots, both those which had been inoculated and the controls, were then placed in paraffin paper bags and held at room temperature for 3 weeks.

The decay developed rather slowly at the temperature of the laboratory during April. After 48 hours only a very small soft black spot had developed around the incisions on the inoculated carrots. No sign of decay had appeared around those on the control carrots. In fact, these 24 incisions closed up rapidly, drying out slightly, and the carrots remained uninfected during the experiment.

Twenty-one days after the inoculations were made, the carrots were removed from the paper bags and observations and photographs were made. Re-isolations from the decayed spots, carried out in the same way as the original isolations from the naturally infected roots, gave pure cultures of the Alternaria that was used for inoculation purposes. Every inoculation of the 84 resulted in infection, the characteristic black rot being produced in all cases. At the end of 3 weeks the decayed spots had developed to about an inch or more in diameter on the surface and half an inch to an inch in depth into the tissues of the root. Each spot was more or less depressed and its surface entirely covered by a

dense black mat of aerial mycelium with conidiophores bearing typical spores, singly or in groups in the manner of fungi generally referred to the genus Macrosporium. The tissues beneath the spot were softened and jet black in color. Plate XI, B, shows a carrot with 3 decayed spots resulting from inoculations. This should be compared with Plate XI, C, a photograph of one of the control carrots, which shows very clearly 2 uninfected incisions. Plate XI, D, is a photograph of median longitudinal section of the inoculated carrot shown in figure B. This last photograph shows the extent to which the fungus developed in the tissues of the root during a period of 21 days at room temperature.

INOCULATIONS OF THE FOLIAGE

During the summer of 1919, field inoculation experiments were carried out, to determine whether this fungus was able to cause a disease of the leaves as well as of the roots. It was also expected that such experiments would enable the writers to determine whether the black rot organism is related to *Macrosporium carotae* Ellis and Langlois, which causes a blight of the foliage.

Inoculations on the Danvers Half-long variety were made during cloudy weather in the last week in June. Spores from pure cultures of the black rot organism on potato agar were mixed with sterilized water and this suspension of spores was then brushed onto the foliage of the plants in two rows of carrots. Eight other rows of carrots in the experimental plot served as controls.

Within two weeks after inoculation, a disease had made its appearance on the inoculated plants. Small discolored areas appeared both on the leaves and the petioles. These spots were at first always brown in color, although in some cases they subsequently turned black. On the petiole the area of dead brown cells extended through the vascular tissues and thus cut off the water supply to the leaf. A wilt of the leaves resulted from these infections on the petioles. They turned yellow, then brown, and the conidia of the parasite developed in large numbers over the entire surface.

During the first month after the disease appeared on the inoculated rows, the control plants in the adjacent rows remained healthy and uninfected. But during early fall the fungus spread to these plants, with the result that the entire plot showed foliar lesions or entirely blighted leaves. The outer leaves of the plants blighted and fell to the ground, forming a mat of dead foliage between the rows.

Isolations were made from leaf lesions and from blighted leaves taken from these infected plants. Two methods were used: (1) spores were washed off in sterilized water and the suspensions thus obtained were plated out in Petri dishes; and (2) pieces of diseased tissues were sterilized by dipping in alcohol followed by mercuric chloride (1–1000), and then

placed on potato agar in Petri dishes. In either case the fungus obtained was similar in every way to that used in making the inoculations. Pure culture transfers were then made from these plates and the fungus was inoculated into carrots, the same precautions being observed against infections from the outside as outlined above for the previous experiments. The typical black rot developed around each inoculation.

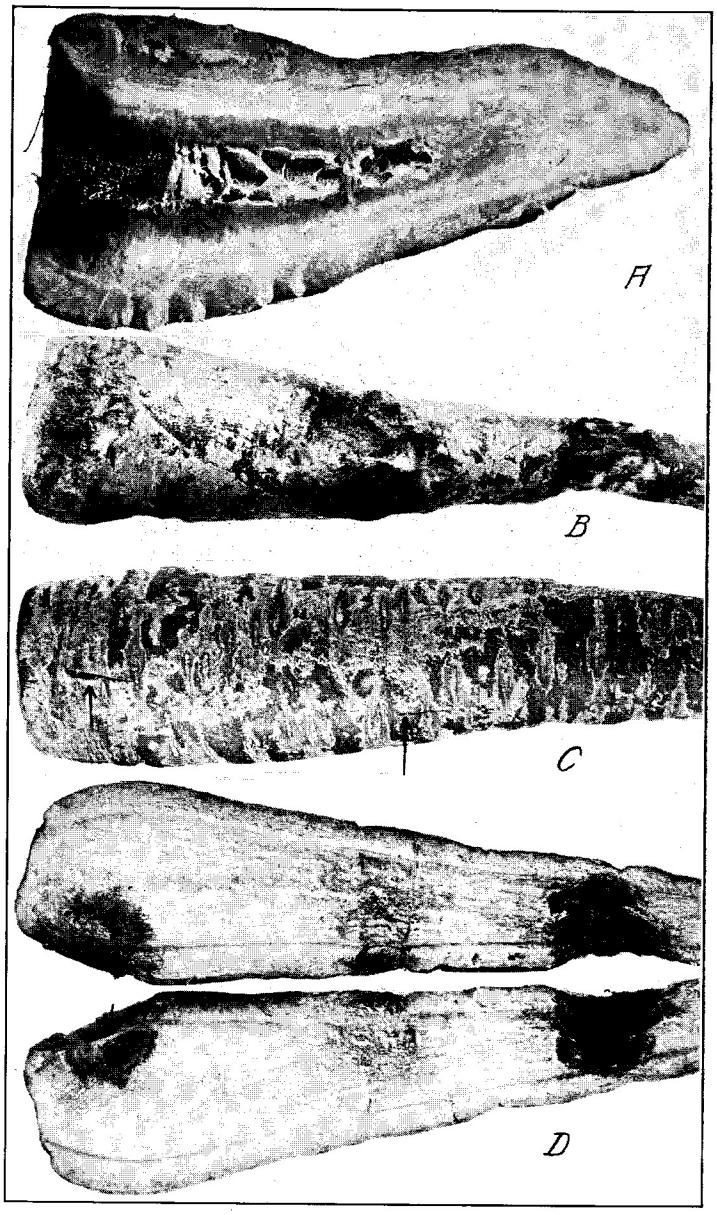
Carrots which showed the blighted foliage were dug up, the tops removed to within an inch of the crown, and the entire surface of the root sterilized with alcohol and mercuric chloride. These roots were then placed in sterilized glass jars and held at room temperature. The same fungus again developed from the bases of the infected petioles and extended down into the center of the root, causing a black rot of the tissues.

Since the infection experiments on the leaves showed that the same organism which causes the black rot of the roots may also cause a disease of the foliage, a careful investigation was made of plants growing in the various fields where the carrots which showed this black rot last winter had been grown during the summer of 1918. Plants showing blighted leaves were collected on the farms at Peconic and Orient, New York, as well as Danvers and South Peabody, Massachusetts, and examined, in the laboratory for the presence of conidia similar to those developed on the inoculated plants. In every case conidia of a Macrosporium were found on the diseased leaves but these conidia did not resemble those developed on the inoculated plants but were readily recognizable as belonging to *Macrosporium carotae* Ellis and Langlois, being characterized by the presence of long, apical sub-hyaline appendages.

Many attempts were made during the summer and fall of 1919 to isolate *Macrosporium carotae* and to obtain pure cultures which might be used for inoculation purposes. While this fungus grew on potato agar and produced typical conidia, the development in culture was very sparse as compared with that of the black rot organism. In fact, the season passed before cultures suitable for inoculation work could be obtained.

THE FUNGUS

The conidial fructifications of the fungus developing on the surface of inoculated carrots, or in pure culture on agar media relatively rich in organic nutrients, as for example, potato dextrose agar, are of the type usually referred to the genus Macrosporium. On the substrata mentioned the production of aerial mycelium is relatively rapid and abundant. From the fuliginous or brownish hyphae that largely compose the dense black mats of aërial growth, the sporophores arise as short branches often several times septate and distinguished by their dark brown color. They bear at the tip usually one or two and more rarely three spores; the latter are attached directly to the sporophore and show no tendency toward a catenulate habit.



BLACK ROT OF CARROTS

A. Longitudinal section of a carrot, showing black rot extending into the core of the root.

B. Three decayed spots resulting from inoculations with *Alternaria radicina*.

C. One of the carrots which were held as controls, showing two incisions which have healed over.

D. Longitudinal median section of the inoculated carrot shown in figure B.

It may be mentioned that when the fungus is cultivated on substrata encouraging a rapid and abundant development, growth comes to a standstill comparatively early, stopping not unusually within 10 to 15 days. Such cessation of growth is, of course, familiar enough to students of nearly every group of fungi, and finds some plausible explanation in the accumulation of toxins. Whatever the explanation may be, the fact is quite obvious that fructifications developing under the conditions described, although often very abundant, are frequently not especially well developed. Thus more satisfactory results can often be obtained by the use of media containing little organic nutrient material on which growth is relatively scant, but which permit the moderate number of sporophores to continue development during a much longer period.

The black rot fungus was accordingly grown on tap water agar and Beijerinck's agar, neither containing any organic nutrients except such as were accidentally introduced. Drying out of the agar was prevented by incubating the Petri dishes in damp chambers. Microscopic examination of the cultures showed that at the end of 60 days the fungus was still growing. The fructifications, while in other details similar to those found on the richer substrata, showed not infrequently a catenulate arrangement of the spores, usually regarded as the distinctive characteristic of the genus Alternaria (Fig. 1, B, C, D,). Proliferation of secondary spores usually occurred at the tip of the primary spores, which were modified to form short subhyaline beaks. Such modification was not observed in spores that had not given rise to secondary spores. Occasionally the secondary structure was produced from one of the segments other than the distal one, in which case a short hyaline lateral process quite similar to the terminal modification supported the secondary spore.

The spores developed on carrots are straight, clavate, ellipsoid, obovoid, or turbinate; light brown to dark brown and dark olivaceous when fully mature; from 3 to 7 times transversely septate, with a longitudinal septum dividing some or all of the transverse segments not occupying a terminal position; measuring usually from 17–20 $\times$ 34–51 μ. When developed on potato dextrose agar the spores generally measure considerably less. On tap water agar or Beijerinck's agar the spore measurements approximate those on the carrot, although showing in general a somewhat greater degree of variability. Spores like those shown in figure 1, A 1–3, probably represent nearly the maximum dimensions attained by the species,—width 22μ (Fig. 1, A 1–3),—length 64 μ (Fig. 1, A 2)—as well as nearly the greatest degree of septation, showing eight cross walls and two longitudinal septa in some of the transverse segments.

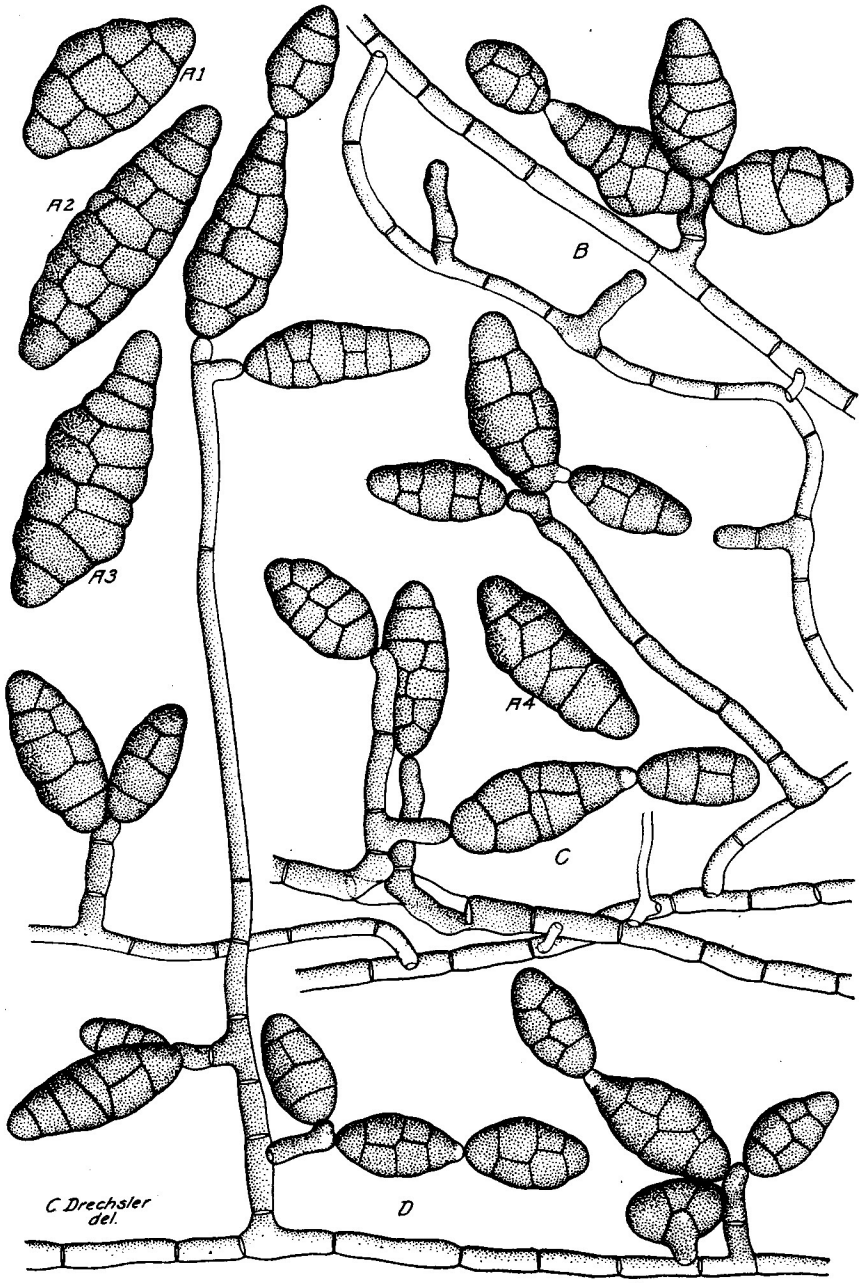


FIG. 1. *Alternaria radicina* from a culture on tap water agar incubated at a temperature of 15 to 20° C. x 535. A, 1–3. Spores illustrating approximate maximum dimensions and approximate maximum degree of septation. A, 4. Spore of average dimensions. B, C. D. Portions of mycelium showing darker colored short sporophores arising from more hyaline hyphae, the catenulate arrangement of some spores, and the tendency of the hyphae to anastomose with each other.

No report could be found in the literature of a root rot of carrots caused by a species of Alternaria, Macrosporium, or any of the allied genera. But Ellis and Langlois[1] described from Louisiana a species of Macrosporium as the cause of a disease of the foliage. This trouble has also been reported from New Jersey, Connecticut, Rhode Island, and other states. The causal organism was described as follows: "On living leaves of *Daucus carota*, to which it is very injurious. St. Martinsville, La., June, 1888, Langlois, No. 1327. Turning the leaves yellow, then brown, black and killing them entirely. Sterile hyphae erect, at first simple, straight, brown and septate, finally somewhat branched above, and 80 to 100 μ high by 4 to 6 μ thick. Conidia clavate, brown, 5 to 7 septate, with one or two of the upper cells longitudinally septate, 55 to 70 μ by 12 to 14 μ slender (1-½ to 2 μ thick), permanent pedicels 80 to 110 μ long."

An examination of type material of *Macrosporium carotae*, as well as of fresh material collected in New Jersey and in Washington, D. C., showed that this fungus is specifically distinct from the Alternaria species found destructive to the root. Figure 2, F 1–11, shows the conpicuous "pedicel" of the conidia of *Macrosporium carotae* developing on the leaves. It may attain a length of 300 μ (Fig. 2, F 4), bear one or two branches (Fig. 2, F 4, 7, 8), and show septa varying in number from one to ten (Fig. 2, F. 6); although more usually much shorter, without branches, and provided with but 3 or 4 septa. The description quoted above would seem to indicate that Ellis considered that portion of the spore designated as the pedicel as the lower end. Since the spore is attached to the conidiophore at its obtuse end, this "pedicel" should be considered as an appendage or prolongation of the terminal cell of the conidium.

In size of spore a much greater difference obtains between the fungus on the root and *M. carotae* than Ellis' description might suggest. The large spores of the latter, exclusive of the appendage, may attain a length of 100 μ or more (Fig. 2, F 1, 2, 4), a width of 30 μ (Fig. 2, F 1, 2), and show from 9 to 11 transverse septa with 2 or even 3 longitudinal septa further dividing the transverse segments. The cultural characters of the two fungi furnish another easy means of distinguishing them, as *M. carotae* produces, on all the kinds of media on which its cultivation was attempted, only very scant growth, and that consisting largely of colorless submerged mycelium; whereas, as has been pointed out, the fungus causing black rot produces considerable growth, both submerged and aerial, grayish black or bluish black, even on media almost devoid of organic food material.

[1] Ellis, J. B., and Langlois, A. B. New species of Louisiana fungi. Journal of Mycology **6**: 35–37. Mar., 1890. *P. 36. Macrosporium carotae*, n. sp.

The writers have never observed any catenulate tendency in the fructification of *M. carotae*. According to Elliott[1], the parasite is to be referred to the genus Alternaria, together with other forms possessing spores with a long beak. It is perhaps not impossible to suppose that if the fungus were grown on substrata more suitable than those employed by the writers, a catenulate habit might be manifested. In rather old material, spores may be found showing one or more short processes that have obviously functioned as sporophores in the production of secondary spores (Fig. 2, E 1–5). In nearly every case, however, such proliferation has apparently been associated with the degeneration of the primary spore, as is evidenced by the collapsed condition of many of its segments. It would appear advisable for the present, therefore, to retain the leaf blight fungus in the genus Macrosporium. The parasite causing black rot of the carrot is assigned to the genus Alternaria and is named *A. radicina* to indicate its relation to the root of its host, to which in nature it seems to be confined.

DIAGNOSIS

Alternaria radicina n. sp.

Aerial mycelium grayish black or bluish black, occurring in extensive mats composed of branching septate hyphae, varying in width from 2.5 to 7.0 μ; the wider hyphae more or less distinctly fuliginous, with constrictions associated with the septa; the narrower hyphae more nearly subhyaline, without perceptible constrictions at points of septation. Sporophores forming the terminations of the larger mycelial filaments, or more frequently arising as lateral branches of the latter at irregular but usually rather wide intervals, and at angles usually approaching a right angle; continuous or one to several times septate; fuliginous to dark brown or brown olivaceous, regularly darker than the axial hyphae from which they originate; when lateral, typically 4–5 × 10–25 μ; developing successively and in close proximity usually 2–3 primary spores, the points of attachment of which after disarticulation are marked by small dark scars. Spores borne directly on sporophores or more tardily in catenulate arrangement, or singly at apex of short sporophoric processes arising from a spore of the preceding order as attenuated prolongation of terminal segment, or as lateral outgrowth of nonterminal, most frequently of basal, segment; typically straight; clavate, ellipsoid, obovoid, or turbinate; light brown to dark brown or dark olivaceous when fully mature; from 3 to 8 times transversely septate, with a longitudinal segment dividing some or all of the segments not occupying a basal or apical position, and more infrequently

[1] Elliott, John A. Taxonomic characters of the genera Alternaria and Macrosporium. American Journal of Botany **4**: 439–476. *Pl. 19, 20.* 1917.

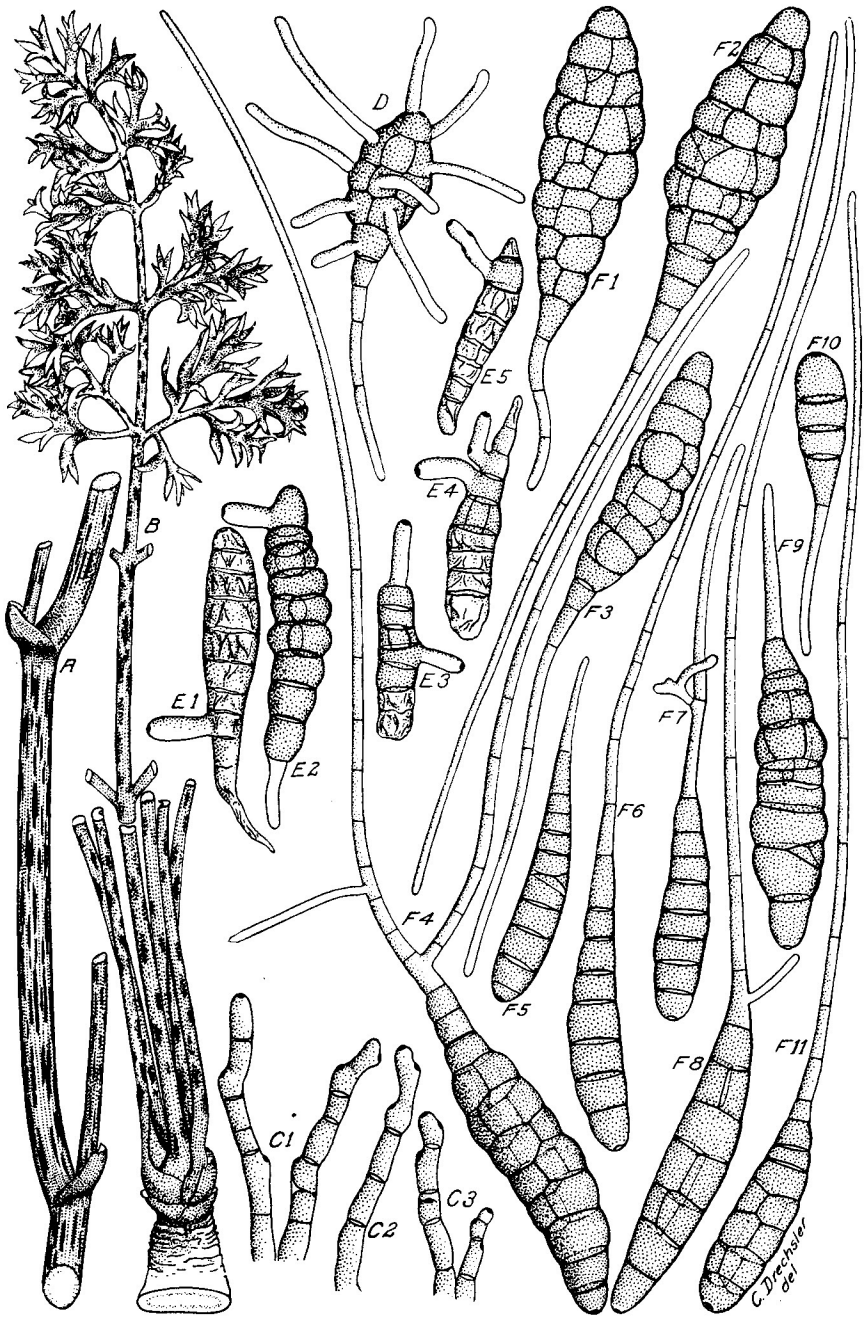


FIG. 2. A. Stem of a mature carrot plant, showing dark longitudinal lesions. × 1. B. Petioles and portion of blade of carrot leaf, showing discolored lesions of blight. × 1. C. Sporophores, showing variation in size and dark scars marking points of attachment of spores. × 350. D. Spore germinating by 9 germ tubes. × 350. E. 1–5. Spores more or less collapsed as result of proliferation of sporophoric processes × 350. F. 1–11 Spores showing characteristic terminal prolongations variable in length, usually septate; and sometimes branching. × 350.

with two longitudinal septa in some of the transverse segments, the septa both transverse and longitudinal associated with constrictions; measuring 10–22 × 34–51 μ, the primary spores usually exceeding those of the second or higher order.

Habitat: Found on roots of *Daucus carota*, in Massachusetts, New York, Pennsylvania, and Washington, D. C., causing a moderately destructive storage decay; also as result of inoculation on foliage and stems, where its attack results in conspicuous spotting and in death of leaves.

DISTRIBUTION OF THE DISEASE

Observations made during the three seasons that have passed since the disease first came to the writers' attention would indicate that the trouble is generally distributed throughout the carrot storage area of the Eastern United States. Dr. J. I. Lauritzen found the disease on carrots obtained from the Washington market, in April, 1919, approximately the same time that it was found by one of the writers on Long Island. During the seasons of 1919, 1920, and 1921 diseased material has been collected at Peconic, Orient Point, Southold, and Mineola, New York, and at Danvers, South Peabody, Arlington, and Framingham, Massachusetts. In March, 1921, the Pittsburg office of the Bureau of Markets Inspection Service sent in characteristic material that was taken from shipments said to have originated in Pennsylvania.

SUMMARY.

1. The "black rot" of carrots, a disease which attacks the roots during storage, is caused by *Alternaria radicina* n. sp.

2. Inoculation experiments show that under very favorable weather conditions this fungus may infect the foliage during growth, causing a spotting accompanied by wilting of the affected leaves. This diseased condition has not been found to occur naturally in any of the localities where the "black rot" of the roots developed.

3. The appearance of this disease on the foliage when it occurs as a result of artificial infection is similar to that produced by *Macrosporium carotae*, a distinct species and obligate parasite producing in nature a widely distributed foliage blight of actively growing carrot plants.

4. *Alternaria radicina* may perhaps best be regarded as a facultative parasite of undoubted vigor affecting mature tissues, particularly those of the more or less bruised or wounded roots while being harvested or undergoing storage.