Reprinted from PHYTOPATHOLOGY, Vol. IX, No. 7, July, 1919

COTYLEDON INFECTION OF CABBAGE SEEDLINGS BY PSEUDOMONAS CAMPESTRIS[1]

CHARLES DRECHSLER

WITH SIX FIGURES IN THE TEXT

That the bacterial black rot of crucifers attacks seedlings more rapidly as well as with more destructive effects than older plants has been noted by several investigators. Smith (2) reports that "When the cabbage is attacked early in the season and severely, it is either destroyed outright in the course of a few weeks, or is so injured that no head forms." And Brenner's (1) observations are particularly pertinent:

"Although cabbage is susceptible to infection with *Pseudomonas*, younger plants, especially seedlings, are destroyed with greater rapidity and certainty than older specimens. Even after eight days the cotyledons show typical symptoms of the disease, which with the increasing growth of the plant, spreads quickly to the first leaves, leading either to complete destruction, or at least to malformation."

Brenner makes no mention of the manner in which the cotyledons of his seedlings became infected; but presumably the young plants were inoculated by needle punctures. It has long been known that outside of wounds the black rot organism gains entrance into older plants by way of the specialized water-pores that occupy serratures on the margins of the leaves. The cabbage seedling, however, does not unfold its first true leaf until a week or more after coming up; and as the cotyledons are devoid of specialized hydathodes, the question arises whether the parasite enters the young unwounded seedling through its roots, or through its stem or cotyledons by a channel not hitherto recorded for this disease.

To solve this question cabbage seeds were planted in greenhouse soil heavily infested with the black rot organism. Over three hundred pots were used in the experiment, each pot being sterilized in the autoclave and

[1] An abstract of this paper appeared in Phytopathology, **4**: 401. D., 1914.

treated with the washings of approximately fifteen grams of steamed potato media thoroughly covered with the yellow slimy growth of the parasite. The pots were kept moderately moist. Germination of the seeds was normal, the young seedlings showing vigorous growth. From twelve to twenty days after planting, the margins of the cotyledons became blackened, the discoloration appearing as a black line extending along the sinus, but never beyond it. A day or two later the affected cotyledons showed a dull streaking toward the center, associated with pronounced symptoms of wilting (fig. 1c); and in another day they had completely collapsed (fig. 1b). In this shriveled condition the cotyledons may remain attached to the stem until the latter also collapses and shrivels. In cases where the first symptoms on the cotyledons appear after one or two leaves have been unfolded (fig. 1d), the lower leaf shows the characteristic dull black streaks and wilts one to three days after the collapse of the cotyledons; and when in one to two days more the younger leaves and the terminal bud become affected, the destruction of the plant is achieved.

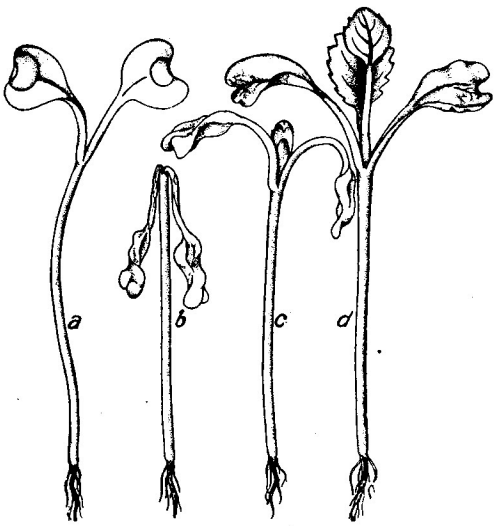

FIG. 1. *a*. Healthy seedling with extruded droplets of water adhering along sinuses of cotyledons. *b*. Diseased seedling in advanced stage of collapse. *c*. Seedling with cotyledons infected and wilted. *d*. Somewhat older seedling with cotyledons beginning to wilt.

A large number of diseased seedlings were examined, and in each case the infection was found to originate at the margin of the sinus of the cotyledon. When material showing very early stages of infection, especially before any macroscopic symptoms were observable, was treated with alcohol to remove the chlorophyll, and the cotyledons examined *in toto* under the microscope, the regions of incipient infection could readily be made out as dark areas always associated with a stoma (fig. 2). Stomata on the edge of the sinus appeared particularly subject to invasion (fig. 3), although numerous substomatal accumulations of bacteria were found near the upper epidermis some distance, usually not exceeding 0.5 mm., back of the sinus.

The progress of the invading bacteria can readily be followed in microtome sections stained with carbol-fuchsin and orange-G; and it differs in

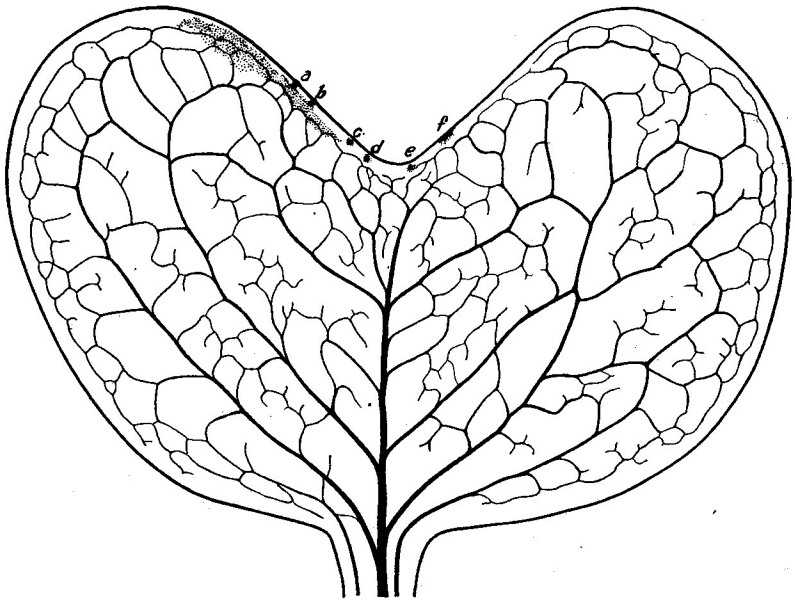

FIG. 2. CABBAGE COTYLEDON SHOWING SIX CENTERS OF INVASION

a b and *f* involving stomata on edge of sinus; *c d* and *e*, stomata back of edge on upper surface

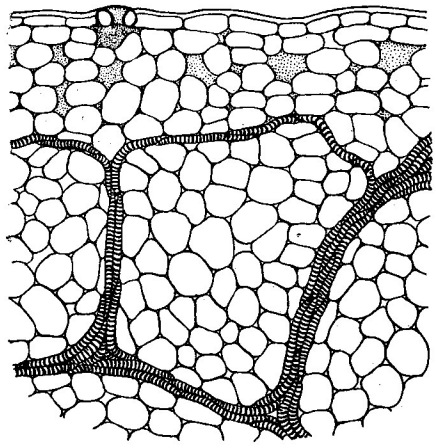

FIG. 3. MEDIAN OPTICAL SECTION OF INFECTED COTYLEDON IN PLANE OF SURFACE, SHOWING BACTERIA ENTERING STOMA *a* IN FIGURE 2

no essential features from the history of water-pore invasions in older plants as described by Smith (3). As a considerable number of stomata are usually invaded, the parasite in spreading from substomatal cavities into the surrounding parenchyma, brings about the coalescence of many centers of infection before a vessel is reached and the disease becomes systemic. The result is that the marginal tissue is usually disrupted along the greater extent of the sinus (fig. 4), yielding the characteristic charring of the edge that constitutes the first macroscopic symptom.

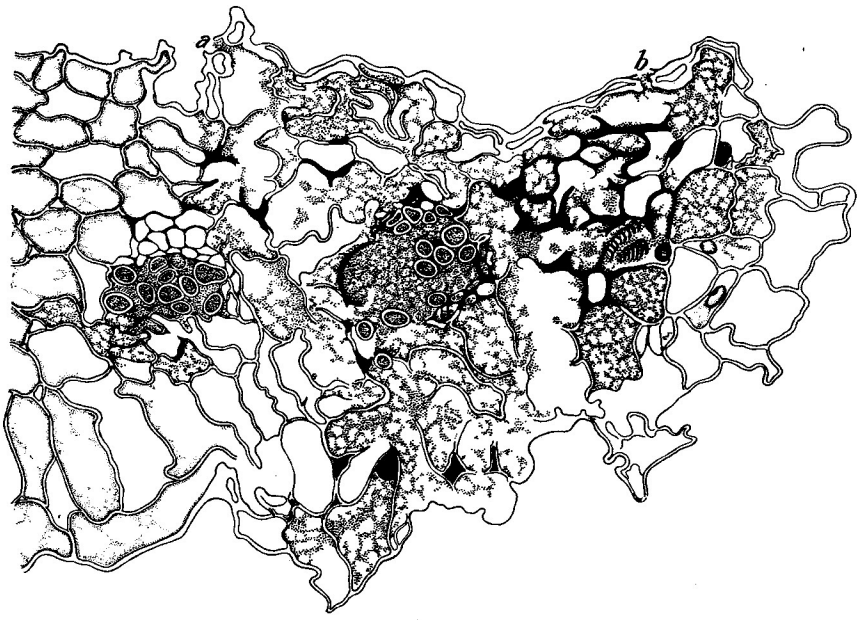

FIG. 4. LONGITUDINAL SECTION OF INFECTED COTYLEDON SHOWING TISSUE IN MARGINAL ZONE ENTIRELY DISRUPTED; AND TWO INVADED STOMATA ON UPPER SURFACE AT POINTS *a* AND *b*

In the stem, the parasite effects its maximum destruction near the attachment of the cotyledons. Cavities develop much more rapidly here than anywhere in older plants, apparently owing to the soft condition of the tissues, and the absence of gaps in the cylindrical stele. Even in seedlings which have merely commenced to wilt (fig. 1c) only a small proportion of xylem elements may be left intact; and a day later when the cotyledons have collapsed, practically all the remaining vessels may be involved in cavities, or occupied by bacteria.

In sections farther down the stem, the cavities become smaller and finally become completely resolved into a number of invaded vessels; and the

invasion of some of these vessels may usually be followed into the upper portions of the root. The decrease in the extent of distribution of the organism downward becomes evident in a comparison of figures 5 and 6, repre-

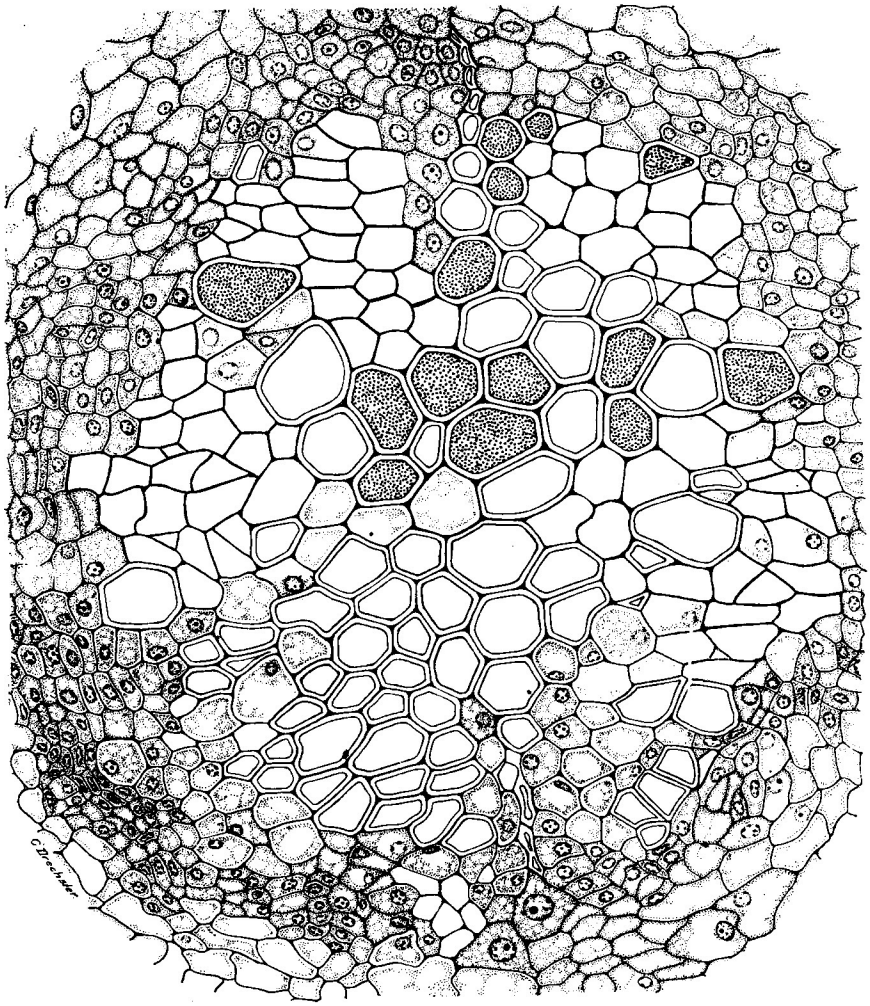

FIG. 5. CROSS-SECTION OF STEM NEAR ATTACHMENT OF DISEASED COTYLEDONS
Most of the xylem elements occupied by bacteria or involved in cavities.

senting conditions found in the same plant in the upper parts of the stem and of the root respectively. In this connection it may be mentioned that although Stewart and Harding (4) believed that the disease can be com-

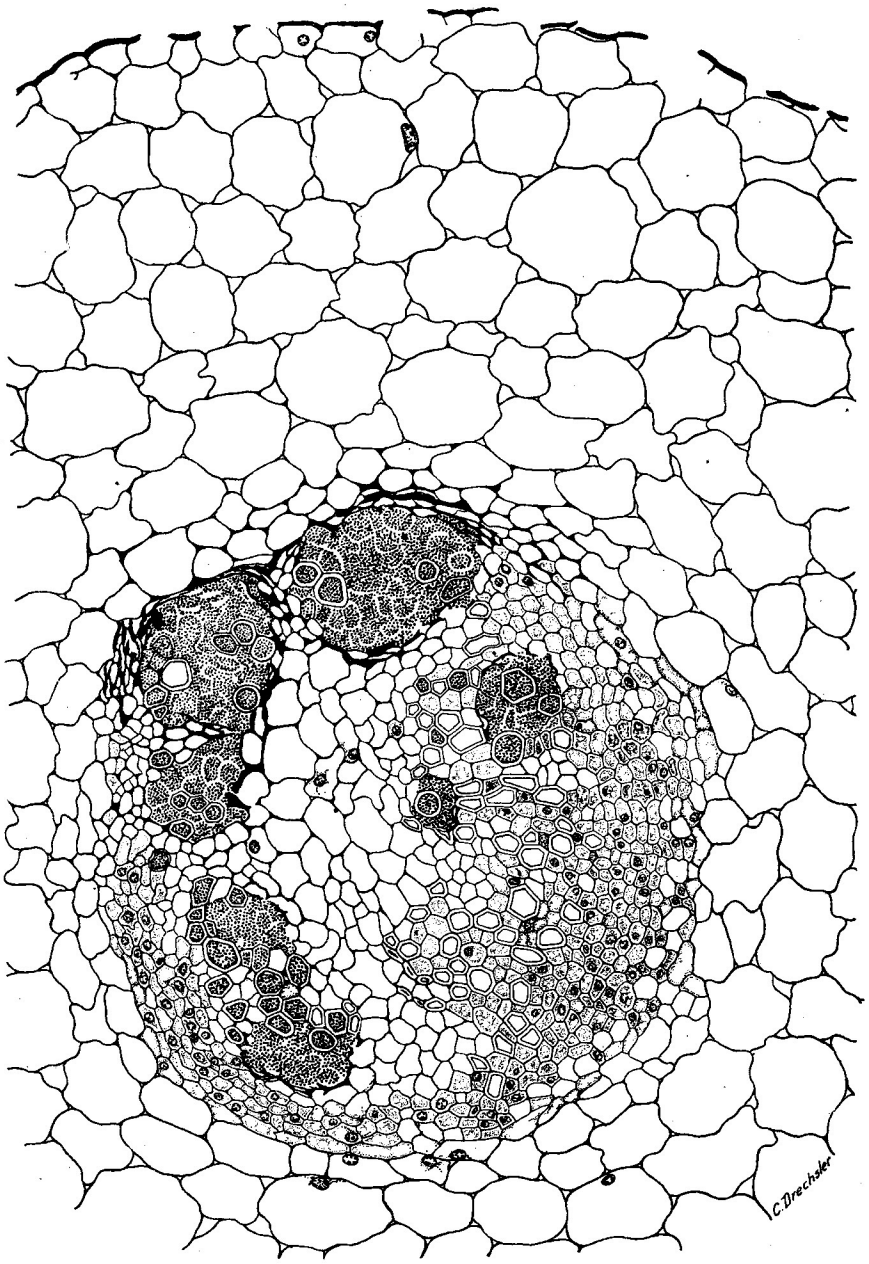

FIG. 6. CROSS-SECTION OF ROOT OF DISEASED CABBAGE SEEDLING USED FOR FIGURE 5, IMMEDIATELY ABOVE ATTACHMENT OF UPPERMOST BRANCH ROOT

Bacterial invasion confined to individual vessels; adjacent cells entirely normal

municated through the root systems, and Smith (2) regarded such a mode of entry as not unlikely in the earlier stages of growth, bacteria could be detected only in the upper portions of seedling roots. And in no case was there any reason to believe that infection had started at the roots, the negative evidence here being in harmony with the observations made on older plants.

The seedling furnishes unusually good material for observations on the possible effects of bacteria on actively functional cells. In every section of the stem numerous vessels plugged with bacteria, may be found adjacent to meristematic cells, parenchymatous cells, or xylem elements in which the nucleus and cytoplasm show no evidence of degeneration. The absence of any signs of injury in such cells as long as the walls remain intact and the cell is not involved in a cavity, is convincing proof that the parasite does not secrete any soluble toxic substance capable of diffusing even very moderate distances.

Since infection through the water pores is dependent upon guttation, it was suspected that perhaps a similar relation might obtain in the case of cotyledons. Accordingly, very young seedlings were placed in a moist chamber where abundant extrusion of water took place within twelve hours. The droplets always appeared at some point along the sinus, adhering either to the reentrant edge or to the upper surface close to this edge—never to the lateral margins or to the central portions of the cotyledons. When these droplets were inoculated by cautiously adding about an equal volume of a suspension of *Pseudomonas campestris*, and the seedlings kept in the damp chamber twelve hours longer, the same symptoms were induced in ten to sixteen days as had been observed in the seedlings grown in the heavily inoculated soil. Over one hundred seedlings were used, and of this number over three-fourths were killed within twenty days after inoculation, the first symptoms always appearing in the region active in guttation, embracing the marginal tissue along the sinus.

A comparison of invaded stomata as well as healthy stomata in the guttating region with those elsewhere situated, has not revealed any morphological differentiation. Probably the former possess merely a miscellaneous capacity to function in the absence of more specialized structures. The physical basis of such localization might be sought, for example, in a possible difference in mechanical response of the guard cells to external conditions; or perhaps, in peculiarities in the nature and disposition of the conducting tissues. As the seedlings grow older, and particularly as the hydathodes of the first true leaves become functional, the amount of water extruded by the cotyledons is, as a rule, greatly reduced; and the possi-

bilities of natural cotyledon infection are reduced in a corresponding measure.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

(1) BRENNER, W.: Die Schwarzfäule des Kohls. Centbl. Bakt. [etc.]. II, **12**: 725–735, 6 fig. 1904.
(2) SMITH, ERWIN F.: Bacteria in Relation to Plant Diseases. **2**: 300-344. Washington, D. C. 1911. (Carnegie Inst. Washington Pub. 27, pt. 2.)
(3) SMITH, ERWIN F.: Pseudomonas campestris (Pammel). The cause of a brown rot in cruciferous plants. Centbl. Bakt. [etc.] II, **3**: 408–415. Ag. 18, S. 10, 1897. 478–486, pl. 6 (col.).
(4) STEWART, F. C., AND HARDING, H. A.: Combating the black rot of cabbage by the removal of affected leaves. New York State Agr. Expt. Sta. Bul. 233: 43–65, pl. 1–2. 1903.