HISTOLOGICAL RELATIONS OF SUGAR-BEET SEEDLINGS AND PHOMA BETAE

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In a former paper it was pointed out that practically all sugar-beet (Beta vulgaris) seed is more or less heavily infected with Phoma betae (Oud.) Fr., and that a large proportion of the seedlings developing from such stock suffer from incipient or severe attack of the fungus, but that under favorable conditions a high percentage of the attacked plants recover sufficiently to make a good growth. It appears that the period during which the sugar beet is susceptible to infection by this fungus is confined to the seedling stage, or, in the case of leaves, to old age, but that when infection has once occurred, it persists. After apparent recovery of the host, the fungus is still present, although it remains concealed until conditions arise sufficiently unfavorable to the beet to enable the parasite to renew its attack. Except in the seedling stage, it seldom accomplishes the immediate destruction of its host, but remains inactive during the first growing season and becomes destructive on mother beets in storage or reappears during the second growing season on the seed stalks or racemes in time to cause infection of the new crop of seed.

Histological studies recently conducted upon seedling sugar beets infected with Phoma betae have shown the fungus fruiting on the surface of young plants that were scarcely past the cotyledon stage. They have also revealed the organism living without serious injury to the host, within the deeper cells of plants that had thrown off the attack and which could safely be predicted to show no further sign of infection during the growing season if reasonably good cultural conditions were maintained. The slides show that the fungus may persist both in and on the tissues of the beet and also indicate something of its modus operandi in attack on seedlings. Sections were prepared from material grown from pasteurized seed in experimental pots in sterilized soil which had been inoculated at the time of seeding with pure cultures of the fungus. The material was controlled by check pots and by recovery of the fungus from certain of the seedlings from each pot as the disease appeared. Damped-off and root-sick seedlings selected at different stages in the progress of the disease and healthy

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seedlings from the control pots were killed in Flemming's solution, embedded, sectioned, and stained with the triple combination in the usual way. Camera-lucida drawings from the slides thus prepared are employed to illustrate this discussion. Most of the seedlings were still in the cotyledon stage, but some that had recovered from the attack had developed their first pairs of leaves. Seedlings which had been entirely killed were so badly disintegrated or so softened by the disease that they did not yield satisfactory material for study. The sections showed the cells in a condition of complete collapse and decay. The cellulose layers of the walls, as well as the middle lamella, were gelatinized and softened to such an extent as to have lost most of their rigidity. The walls were broken and fragmented, but this may have resulted from handling during the process of washing and dehydrating. Bacteria were present, of course, and the softening of the walls, which made them so liable to fracture in handling, may have been due in part to the action of these agents.

Cells of badly diseased but still living seedlings presented more favorable material for studying the histological relations of the parasite and host. The cells were often nearly filled with the fungus, which showed a tendency to remain within the cell rather than in the middle lamella, although it frequently penetrated the walls (Pl. I, fig. 1). Now and then a thread of the fungus was observed running between the cells for a little distance, but the indications are that, while the organism dissolves the middle lamella, it does not feed upon it. Heavily invaded cells are consumed, the cytoplasm disappears, and the nuclei disintegrate. The middle lamella gelatinizes, so that the cellulose lamellae may become widely separated while the cellulose layers are broken and disintegrated or even dissolved (Pl. I, fig. 2). The first visible indication of the alteration in the walls is a change in their reaction toward the stain. They take the safranine more deeply and retain it more tenaciously than do the walls of normal cells. With the progress of the disease a border area of increasing width, which also takes the safranine deeply, develops on either side of the walls, as if the substances which retained the dye were gradually diffusing from the wall and spreading into the surrounding space.

In cases of less serious infection, where recovery is possible, or in tissues which have just been invaded, a somewhat different condition exists. Plate I, figure 3, represents a recently invaded portion of a rather badly diseased seedling which would probably have been unable to recover. The cell walls show the gelatinized condition only in a moderate degree and in an area confined to the points where it has been penetrated by the mycelium. The mycelium has expanded in one of the cells in a manner not frequently noted, and the effect of the parasitism is apparent in the abnormal condition of the host nuclei. Evidence of disease was sometimes manifested in the neighboring uninfected cells of such mate-
trial by the unusual appearance of the nuclei. Dumb-bell forms, budding, and indirect division were observed occasionally, but never in any large number (Pl. I, fig. 4, 5, 6).

The most interesting phenomena in many respects, as well as the most puzzling, are those associated with recovery and healing. Sugar beets attacked by the fungus frequently send out new side roots from a point above the invasion and succeed in preventing the destruction of this new growth. Cases were common in which the region invaded and dis-integrated had been confined to the outer tissue. The central vascular region and the surrounding layers of cells resisted the attack and eventually succeeded in sloughing off the killed tissue. The fungus was frequently found developing its pycnidia on the killed portions of such recovering seedlings, while the host tissue, only a few cells below, appeared perfectly normal (Pl. II, fig. 1).

The most striking thing brought out by a study of the sections, however, is the presence of the fungus apparently established in a condition of reduced relative virulence in the interior tissue of beets which have recovered from the attack and which are assured of making a good growth (Pl. II, fig. 2). In such cases even the invaded cells are not killed, and the adjacent ones appear perfectly normal in every respect. So far as has been observed, the cells thus invaded are adjacent to vascular tissue, but the organism has never been seen in the conducting elements. The infection is confined to a vertical chain of cells, and in no case was more than a single unbranched hypha observed.

The physiological relation here presented is an exceedingly interesting one and its investigation is of the highest scientific and practical importance.

It is difficult to explain just how an organism capable of producing such complete collapse in cells of seedlings should suddenly find its action checked and confined to a saprophytic existence on an area of discarded surface tissue, but the means by which it establishes itself within the highly nutritive living cells of the interior and is at the same time compelled to remain in a quiescent condition is still more problematical. The condition presents a relatively highly developed type of parasitism in which the organism voluntarily or by compulsion permits the completion of the normal life history of the host while securing for itself the assurance of perpetuation through infection of the seed. The balance, however, is not a perfect one, since, if the host encounters sufficiently adverse conditions during either of the growing seasons or in storage, the activity of the parasite is renewed and the sugar beet is destroyed, thus preventing seed production and the perpetuation of the parasite through the seedling channel.
Fig. 1.—Section of a sugar-beet seedling invaded by *Phoma betae*, showing distribution of the mycelium and the action of the fungus on the protoplasm and cell walls. \( \times 530 \).

Fig. 2.—Section of sugar-beet seedling showing characteristic action of *Phoma betae* on the cytoplasm and nuclei and cell walls in cases of serious infection. Note the gelatinized condition of the middle lamella. \( \times 530 \).

Fig. 3.—Section of sugar-beet seedling showing *Phoma betae* penetrating the cell walls and expanding in one of the cells. The nuclei show signs of degeneration. \( \times 530 \).

Fig. 4, 5, and 6.—Abnormal nuclei from uninfected cells adjacent to invaded tissue of sugar-beet seedlings. The nucleus in figure 6 appears to be in the process of direct division. \( \times 1,330 \).
PLATE II

Fig. 1.—Section through a sugar-beet seedling which has recovered from an attack of *Phoma betae*, showing a young pycnidium of the fungus forming on the discarded, killed tissue. × 500.

Fig. 2.—Longitudinal section through a sugar-beet seedling which had recovered from an attack of root sickness due to *Phoma betae*, showing the presence of the fungus established in a condition of reduced virulence in the living cells. × 530.