THE MIGRATION OF BACILLUS AMYLOVORUS IN THE TISSUE OF THE QUINCE[1]

By HERBERT A. WAHL[2]

Instructor in Botany, Pennsylvania Agricultural Experiment Station

HISTORICAL REVIEW

The fire-blight organism, *Bacillus amylovorus* (Burr.) Trev., is of considerable economic importance because of the disease it produces on three hosts—apple, pear, and quince. A study of the comparative cytological reactions of host and parasite for these three species is of particular biological interest.

Nixon (*5, p. 9*)[3] first described the annual life cycle of *Bacillus amylovorus* in apple tissue. According to this author, the life cycle consists of two stages. The first stage is characterized by the formation of zoogloeae in which the bacteria migrate intercellularly, with the formation of schizogenous cavities "by toxic plasmolysis and crushing." The regions invaded by the bacteria during this stage are described as follows:

* * * the "optimum path" of migration is confined to the intercellular spaces of a zone of cells in the inner cortex * * *. From the "optimum region" the invasion of adjacent, less favorable tissues, may follow * * *. The last tissues to be invaded are the cambium, the xylem and pith, and these are rarely seriously attacked.

In the second or "pseudo-fructification" stage the bacteria become smaller and invade the cells by dissolving the cell wall, thus forming lysigenous cavities. The second stage culminates in the formation of cysts within the cells, in which condition the organism passes the winter.

Haber (*2, p. 10*) found that under artificial conditions young apple leaves may be the portal of infection; that the bacteria migrate in the form of zoogloeae in the intercellular spaces of the vein parenchyma and spongy mesophyll of the leaf, and that death of the tissues is caused by "plasmolysis of the protoplast, the collapse of cell walls, and the separation of contiguous cell walls to form schizogenous cavities."

The results of Gibbons's[4] investigation concerning the invasion of the organism and its effect on the host tissue in pear paralleled Nixon's results with apple. Tullis (*7*) also corroborated Nixon's work as to the general features of the migration of the organism.

Rosen (*6*), in studying the invasion of the floral structures and petioles of pear and the stems of apple and pear, also reported migration as taking place in the spaces between the cortical cells. The middle lamellae and the cell walls of these cells are broken apart by

Received for publication July 10, 1931; issued August, 1932. Contribution from the Agricultural Experiment Station of the Pennsylvania State College, No. 559, and from the Department of Botany, No. 76.

[2] The writer wishes to express his appreciation for the assistance and helpful suggestions of Dr. E. L. Nixon, under whose direction these investigations were conducted. He is also grateful to Dr. H. W. Thurston and others for valuable suggestions in the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 63.

[4] GIBBONS, F. P. THE MIGRATION OF BACILLUS AMYLOVORUS IN PEAR TISSUE. (Unpublished master's thesis, Pennsylvania State College.)

chemical dissolution with no signs of pressure observable on the cells. This dissolution is noted as occurring ahead of the invading mass of bacteria. The movement of the mass of bacteria in the intercellular spaces is considered "a passive one engendered by the accretion of great numbers of bacteria rather than an active pseudopodlike movement * * *." Rosen reports that all the stem tissues are later invaded and that death of the host is attributable to the formation of cavities by dissolution, particularly in the phloem and cambium, rather than to toxic products secreted by the pathogene.

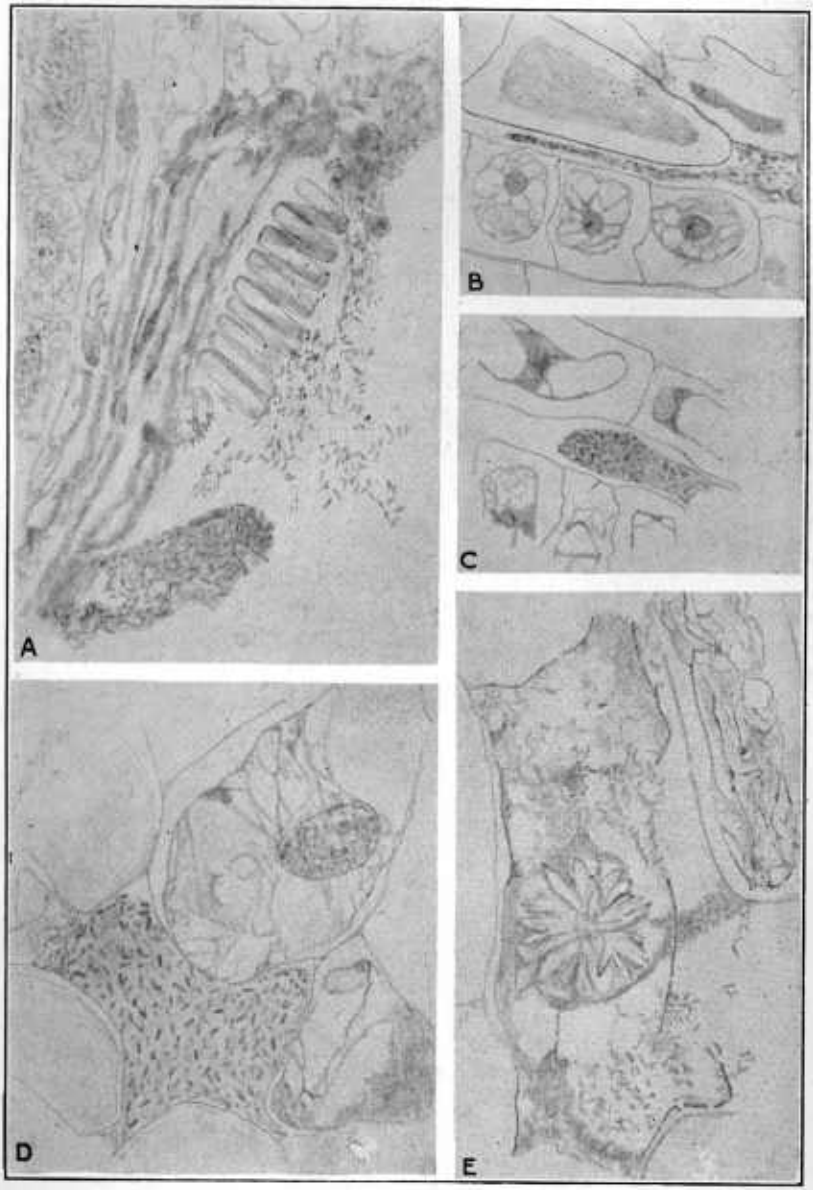
A review of the literature reveals no reference to any microscopic studies of the invasion of the tissues of the quince (*Cydonia vulgaris* Pers.) by this organism.

METHODS

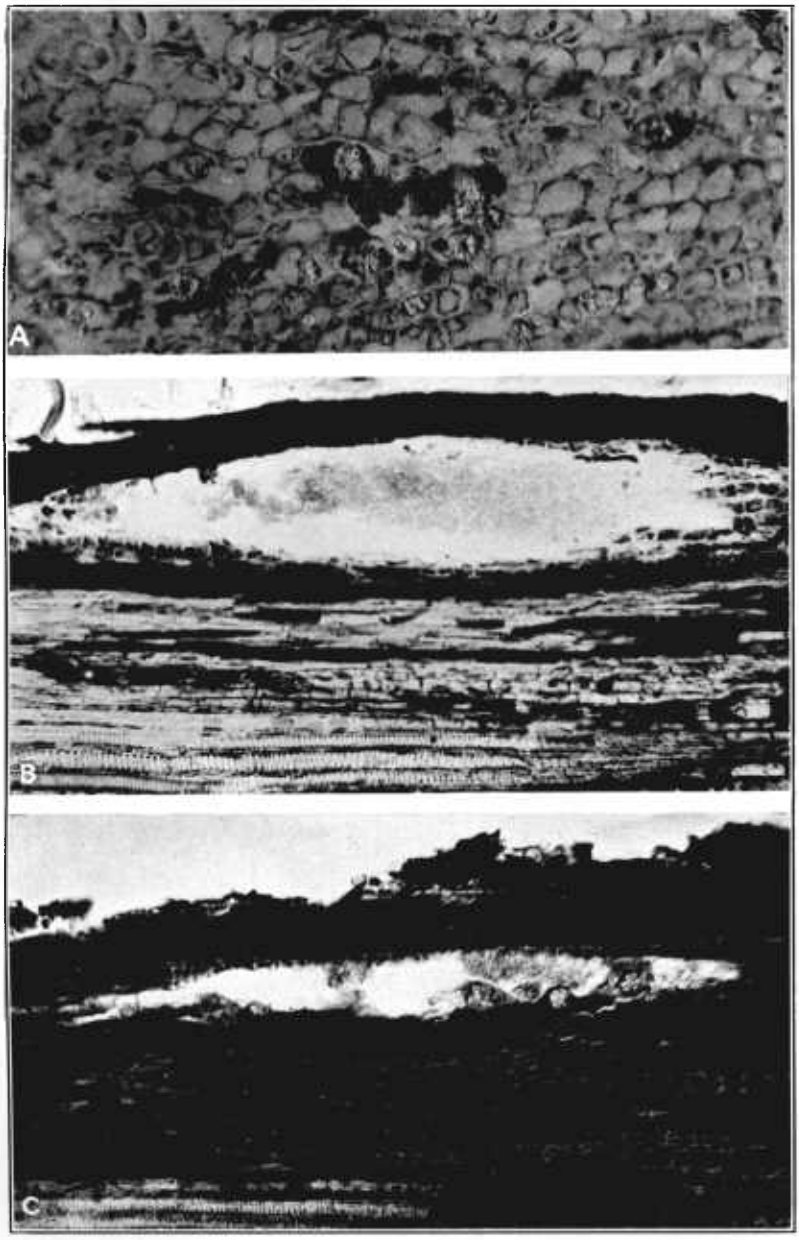
The research presented in this paper was begun at the Pennsylvania State College in the spring of 1925, when a series of inoculations was made on young quince trees growing in the college greenhouse. A pure culture of *Bacillus amylovorus* had been secured previously by isolating the bacteria from blight cankers that had been brought into the laboratory and kept in a moist chamber until the gummy exudate characteristic of this organism appeared. The culture was grown on Lima bean agar and its pathogenicity proved by inoculation of quince trees and reisolation of the organism. Inoculations were made with a sharp laboratory needle introduced at the growing tip of the twig, the puncture being made entirely through the twig. Sections were taken after $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 4, 6, 8, 10, 12, 16, 18, 24, and 48 hours, fixed in Flemming's weak solution, and embedded in paraffin by the usual method. In the spring of 1927 a duplicate series of inoculations was made on quince trees growing in the college orchard. The method of inoculating and embedding was the same as that used in the earlier experiments except that sections were taken up to 142 hours after inoculation and were fixed in Flemming's weak, Petrunkevitch's, and alcohol-formalin-acetic fixing fluids. Sections were cut 9μ to 11μ thick and stained with Flemming's triple stain.

OBSERVATIONS

Microscopic examination of the early stages following inoculation showed the bacteria lying along the edge of the puncture, where they had multiplied rapidly in the broken remains of the injured cells. Invasion of the uninjured tissues was found to have taken place in a narrow cylindrical area in the inner cortex extending around the stem about 10 cells inward from the epidermis and several cells outward from the phloem region. In this area the bacteria were found in zoogloeal masses invading the intercellular spaces in all directions from the puncture, though more rapidly in a longitudinal than in a tangential direction. Radially the intercellular spaces were occupied only in a layer three to five cells wide. No invasion beyond the puncture was observed one-half hour after inoculation, although at that time large masses of bacteria were present in the puncture. (Pl. 1, A.) After 4 hours the bacteria had reached a distance of about 0.037 mm from the puncture (pl. 1, B), and after 12 hours they had reached a distance of 0.5 mm (pl. 1, C). At this stage none of the cells in the infected area were occupied nor were the cell contents in any way injured, except for a slight plasmolysis.



A.—Bacteria in the puncture, one-half hour after inoculation. The broken remains of cells, including a xylem vessel, are present. × about 1,750.
B.—Zoogloeal mass formed at edge of puncture invading intercellular spaces of inner cortical region, four hours after inoculation. × about 1,000.
C.—Tip of zoogloea, 12 hours after inoculation. ×-about 1,250.
D.—Bacteria occupying a space between cortical cells with evidence of slight pressure and splitting of cell walls, 42 hours after inoculation. × about 1,750.
E.—The beginning of lysigenous cavity formation by the dissolution of cell wall and protoplasm. The crystal in the cell is not attacked by the bacteria. × 1,000.



A.—The formation of a lysigenous cavity by the dissolution of adjacent cortical cells; 110 hours after inoculation at a distance of 2.3 cm from puncture. × about 185.

B.—An extremely large lysigenous cavity in the cortex; 66 hours after inoculation adjacent to puncture. All the cortical cells are dead, but there is no noticeable effect on phloem, cambium, xylem, or pith cells. × about 185.

C.—Mostly tangential section of cortex showing death to all cells; 142 hours after inoculation adjacent to puncture. × about 185.

After 42 hours the bacteria had traveled a distance of about 1.5 mm from the point of entrance and had formed numerous, though not large, schizogenous cavities. (Pl. 1, D). Certain cells were split apart at the middle lamella, and the cell walls were slightly invaginated. Also, during this time and in the same region, a few cells were seen to be entirely filled with bacteria. These cells retained their normal outline, while the protoplast was not destroyed but was pushed to one end of the cell, where it became an almost or quite homogeneous mass. Although the complete life cycle of *Bacillus amylovorus* is not being considered here, this condition corresponds closely with the early stages shown by Nixon in apple in which the bacteria multiply rapidly within the lumen of a cell, eventually rounding up to form a cystlike body that is the overwintering form of the organism.

After a time the organism apparently changes its method of invasion and migrates without regard for cells or intercellular spaces. In this stage cell walls and protoplasts alike succumb to the invading horde of bacteria. Cells are entirely broken down, so that no indication of their previous existence remains. The bacteria are now arranged in thin radiating strands in the lysigenous cavities thus formed, their arrangement suggesting that they have utilized the cell wall, nucleus, and cytoplasm for food and have thus formed a strand of bacteria where once there was a strand of cytoplasm or a cell wall. This takes place within 96 hours after inoculation, when the bacteria have traveled a distance of 1 to 1.5 cm from the puncture, and continues until the entire inner cortical region is broken down, the cells shrunken, and their protoplasts entirely disorganized. (Pls. 1, E, and 2, A, B, C.) This is the condition at 142 hours after inoculation, when the invasion has extended longitudinally from the puncture at least 5 cm. Stages later than 142 hours after inoculation have not been studied.

DISCUSSION

The results secured in the present study agree with those of Nixon (*5*) and Haber (*2*) in their work with apple and of Gibbons [5] with pear, thus showing that there is to be found no essential difference in method of migration in any of the three economic hosts of this parasite. These studies also show essential agreement with the work of Hill with *Bacterium tumefaciens* in tomato (*3*) and *Bact. tabacum* in tobacco (*4*) and of Beach with *Bact. vignae* in Lima bean (*1*), so far as the method of migration of the bacterial pathogene is concerned.

The zoogloeal mass invaded the tissues at an average rate of 9μ per hour for the first 4 hours, 42μ per hour for the first 12 hours, 36μ per hour for the first 42 hours, 156μ per hour for the first 96 hours, and 352μ per hour for 142 hours. These figures represent only one to several measurements in each case. Although the figures are open to error because of the small number of measurements and because of the difficulty of determining the exact extent of the invasion, a marked difference in rate is shown between the earlier and later stages. Thus, for the 38 hours from the 4 to the 42 hour period after inoculation, the average rate of migration was 38μ per hour. During the next 54 hours the average rate had increased to

[5] GIBBONS, F. P. Op. cit.

about 250μ per hour, and the average for the next 46 hours was 761μ per hour. Haber found that the zoogloeae in apple leaves traveled a distance of 0.5715 mm in 12 hours and had reached a distance of 1.5 mm from the puncture after 24 hours. This is an average rate of 47.6μ per hour for the first 12 hours and 62.5μ per hour for the first 24 hours. Nixon reports a migration of 3 or 4 inches in 24 to 48 hours after inoculation in apple stems. Four inches in 48 hours represents a rate of 2,115μ per hour. It would seem desirable that further work be done with the different hosts under controlled conditions of temperature, humidity, age, and rate of growth of host, etc., to determine, if possible, the factors responsible for such a wide range in the rate of migration.

Nixon found that the region of intercellular migration in apple may extend entirely around the stem and, longitudinally, may involve the entire twig and adjacent limb, and under optimum conditions may extend several feet. In this study the bacteria were found entering the cells of quince within a distance of 1.5 cm from the point of inoculation, and were not found invading the entire circumference of quince stems through the intercellular spaces.

During this intercellular migration some cells in the path of the invading mass of bacteria become separated along the line of the middle lamella. How this separation takes place is still problematical. Two possibilities, however, present themselves as primary factors—either the pressure from the mass of bacteria is great enough to separate the cells or some solvent action of the products of the organism causes the dissolution of the middle layers of the cell wall. Slight evidence of pressure exerted upon the cell is present in the form of indentations in the cell wall. (Pl. 1, D.) Since, however, the cells all through the region of intercellular migration and schizogenous cavity formation are slightly plasmolyzed, it is evident that the cell turgor in these cells is quite low or is, perhaps, entirely lacking. Thus very little pressure would need to be exerted by the bacterial mass to cause invagination of the cell wall. The evidence, then, indicates that only a very slight amount of mechanical pressure is exerted by the invading mass of bacteria. This leads to the conclusion that the cells are split apart by some solvent action of the bacteria.

Many stages in the dissolution and disorganization of cells resulting from bacterial activity were found. Plate 1, E, shows the bacteria at work in the destruction of the wall and contents of a single cell. In Plate 2, A, may be seen an early stage in the formation of a lysigenous cavity by the breaking down of adjacent cells. Advanced stages of these processes are shown in Plate 2, B and C, where extensive cavities have been formed by the total disintegration of several layers of cells in the most susceptible region in the inner cortex. The lysigneous cavity thus formed (pl. 2, A) measures 80μ by 425μ. By examining serial sections this cavity was found to extend in a tangential direction around the stem at least 150μ, each section showing the cavity occupied by a mass of bacteria. The preparations contained many such cavities, each showing the bacteria in good condition. This would seem to contradict the statements of Tullis (*7*) and Rosen (*6*) as to the inadequacy of stained paraffin sections for preserving the correct condition and relation of *Bacillus amylovorus* and its host.

There seems to be no reason to doubt that the cell walls and contents are acted upon by growth products of the bacteria and broken

down just as other food materials are broken down by other bacteria. The finer details of the dissolution of the host cells and the exact nature of the method by which the bacteria produce the dissolution are not within the scope of this investigation. Far more important are the reasons for the death of all the tissues, even those not directly attacked by the organism, by the time the organism has been active in the stem for 100 hours. The organism was not found in any tissues of young quince stems except the narrow cortical region previously described. In spite of this, tissues, including phloem, cambium, and pith, are definitely shown to be dead adjacent to the area of cortical invasion. (Pl. 2, C.)

These results are in contrast to those reported by Rosen (*6*), who states that in apple stems "* * * by far the most serious effects on the infected twig or limb result * * * from invasion of the phloem and cambium. It is the destruction of the latter and not the cortex which results in the death of the twig or limb." Rosen states further (*6, p. 64*) that "* * * the evidence is indeed substantial for the assumption that diffusible toxic products are not produced by *B. amylovorus*." If Rosen's results are correct, then we are confronted by the fact that the same organism must operate in an entirely different manner to produce the results here recorded for quince. Some tissues of quince stems are killed without being invaded by the organism. The tissues that are invaded are not those directly concerned with either growth or food conduction. The destruction of the tissues invaded is not sufficient to explain the death of the entire twig. Therefore, the evidence is substantial for assuming that diffusible toxic products of *Bacillus amylovorus* are responsible for the death of the stem tissues of quince not invaded by the organism.

SUMMARY

Bacillus amylovorus migrates through the intercellular spaces of the inner cortex of quince in the form of zoogleae.

During this invasion schizogenous cavities are produced in quince in a manner similar to that previously reported by Nixon in apple.

Intracellular invasion of the cortex, involving formation of lysigenous cavities, occurs within 96 hours after inoculation.

Death of all the stem tissues occurs within 100 hours after intercellular invasion and within 48 hours after intracellular invasion of the adjacent cortex, although during this time the organism is present only in the cortex.

LITERATURE CITED

(1) Beach, W. S.
1928. The relation of Bacterium vignae to the tissues of lima bean. Penn. Agr. Expt. Sta. Bul. 226, 15 p., illus.

(2) Haber, J. M.
1928. The relationship between Bacillus amylovorus and leaf tissues of the apple. Penn. Agr. Expt. Sta. Bul. 228, 15 p., illus.

(3) Hill, J. B.
1928. The migration of Bacterium tumefaciens in the tissue of tomato plants. Phytopathology 18: 553–564, illus.

(4) ———
1929. Migration of Bacterium tabacum through the leaf tissues of Nicotiana tabacum. (Abstract) Phytopathology 19: 97.

(5) NIXON, E. L.
1927. THE MIGRATION OF BACILLUS AMYLOVORUS IN APPLE TISSUE AND ITS EFFECT ON THE HOST CELLS. Penn. Agr. Expt. Sta. Bul. 212, 16 p., illus.
(6) ROSEN, H. R.
1929. THE LIFE HISTORY OF THE FIRE BLIGHT PATHOGEN, BACILLUS AMYLOVORUS, AS RELATED TO THE MEANS OF OVERWINTERING AND DISSEMINATION. Ark. Agr. Expt. Sta. Bul. 244, 96 p., illus.
(7) TULLIS, E. C.
1929. STUDIES ON THE OVERWINTERING AND MODES OF INFECTION OF THE FIRE BLIGHT ORGANISM. Mich. Agr. Expt. Sta. Tech. Bul. 97, 32 p., illus.

U. S. GOVERNMENT PRINTING OFFICE: 1932