Histopathology of Chrysanthemum Stems Artificially Inoculated with Erwinia chrysanthemi

Barbara White Pennypacker, Paul E. Nelson, and Robert S. Dickey

Research Assistant and Professor, respectively, Department of Plant Pathology, The Pennsylvania State University, University Park 16802; Professor, Department of Plant Pathology, Cornell University, Ithaca, New York 14850.

Contribution No. 771, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication as Journal Series Paper No. 4619. Authorized for publication 5 February 1974.

Accepted for publication 18 May 1974.

ABSTRACT

Chrysanthemums, cultivar 'Giant #4 Indianapolis White,' were inoculated in a young leaf midrib with a chrysanthemum isolate of *Erwinia chrysanthemi* and placed in a mist chamber. The stems of these plants were examined histologically at selected intervals during disease development. The pathogen caused extensive breakdown of xylem parenchyma, pith, and phloem tissue, as well as separation of cells in the xylem and pith. Cell walls in the affected areas were frequently thinner than the walls of cells in nonaffected areas. *Erwinia chrysanthemi* moved vertically in the host through xylem vessel elements and some limited vertical movement probably occurred in the pith tissue. Vascular plugs of pectic substances and bacterial masses occasionally were present in the xylem vessel elements, but mechanical rearrangement of xylem vessel elements and subsequent interruption of the transpiration stream, appeared to be a major cause of the wilt symptom.

Phytopathology 64:1344-1353.

Additional key words: *Chrysanthemum morifolium*, bacterial blight, pathological anatomy.

Bacterial blight of the florist's chrysanthemum *(Chrysanthemum morifolium)* was discovered in New York in 1950 and has subsequently been reported in other chrysanthemum-growing regions of the USA and Canada (5, 6). Symptoms of bacterial blight in chrysanthemum consist of discoloration and water soaking of the stem. The discolored area of the stem eventually becomes hollow, ruptures, and collapses. The stem crack reveals dark brown, disintegrating tissue and bacterial ooze in the interior of the stem. Leaves above the affected region wilt soon after the first stem symptoms appear and occasional externally visible brown streaks extend into the petiole and leaf veins. Brown streaks are common in the vascular tissues of the stem and may extend to the soil line. The disease is favored by high temp and high humidity (5). Burkholder et al. (5) isolated a nonspore-forming, gram negative, rod-shaped bacterium with peritrichous flagella from plants showing these symptoms and proposed the name *Erwinia chrysanthemi* for the bacterium causing bacterial blight of chrysanthemum.

This study was initiated in an attempt to determine whether the bacterium is systemic in the vascular system of chrysanthemum plants as implied by Burkholder et al. (5). The anatomical manifestations of the host-parasite relationship were also examined in an attempt to determine the actual mechanism of pathogenesis.

MATERIALS AND METHODS.—Rooted cuttings of *Chrysanthemum morifolium* Ramat. 'Giant #4 Indianapolis White' (Yoder Brothers, Inc., Barberton, Ohio) were potted in 10.2-cm (4-inch) diam pots of a steam-treated 1:1:1 mixture of peat:perlite:soil. The pots were placed on steam-treated wooden blocks on a greenhouse bench to promote good drainage and lessen the possibility of contamination from the bench. The plants were kept under continuous light to prevent floral initiation.

After 10 days of growth, the plants were inoculated with isolate EC-16 of *E. chrysanthemi*. Thirty-nine chrysanthemum plants were inoculated by stabbing the midrib of the third youngest leaf with a fine sewing needle smeared with cells of *E. chrysanthemi*. The plants were ca. 110 mm tall at the time of inoculation. The bacterial culture used for inoculation was grown on slants of Difco nutrient agar, transferred twice at 48 h intervals, and used as inoculum when the second transfer was 48 h old. The inoculation method was selected to satisfy the need for the inoculation of the pathogen into a specified tissue, i.e. vascular tissue, rather than unspecified tissue which occurs when the main stem is directly punctured with a needle containing bacterial cells. Inoculation times were selected so plants could be sampled at predetermined intervals up to 96 h after inoculation. Five uninoculated check plants were stabbed as described, using only a sterile needle. Following inoculation, all plants were

placed in a mist chamber in a greenhouse. The chamber had a temp range of 25 to 37 C with a relative humidity (RH) of 100% as determined by wet bulb/dry bulb copper-constantan thermocouples, and provided conditions favorable for development of bacterial blight (5).

Plants were sampled at 2, 4, 6, 8, 10, 12, 16, 24, 32, 48, 56, 72, and 96 h after inoculation. Each sample consisted of the stems of three inoculated plants minus roots and leaves. One check plant was sampled each 24 h period. The outlines of the chrysanthemum stems were then traced on lined paper, and the stems were surface sterilized in a 10% solution of Clorox (5.25% sodium hypochlorite) for 5 min, drained on fresh paper towels, and cut into numbered pieces 5 mm long with sterilized razor blades. The odd numbered pieces were fixed in Rawlin's formalin-aceto-alcohol solution No. 1 (FAA) (13). The even numbered pieces, starting with the 12 h sample, were placed in tubes of Difco nutrient broth and examined after 10 days, at which time the presence of any bacterial growth was recorded on the corresponding stem drawing.

After 10 days' incubation at 21 C, a transfer loop of liquid from each nutrient broth tube showing turbidity was streaked onto a petri plate of nutrient agar. After sufficient bacterial growth was present, individual colonies of all bacteria morphologically resembling *E. chrysanthemi* were picked from the nutrient agar plates and streaked onto nutrient agar slants. After ca. 48 h of growth, the bacteria thus selected were tested on fresh slices of potato tuber tissue in individual petri dish moist chambers which were prepared by placing 5 ml of sterile distilled water on a 9-cm disk of filter paper. The 5- to 10-mm thick potato slices were taken from sound, washed tubers which had been surface sterilized as described previously. Each potato slice was streaked with bacterial cells and stabbed through the bacterial smear ca. 10 times with a transfer needle to wound the potato cells. The potato slices were examined after 48 h for soft rot (5). In addition, bacteria found to be capable of rotting potato tuber tissue were also tested for their ability to incite bacterial blight symptoms in chrysanthemum. The chrysanthemum pathogenicity test was conducted as described for the original chrysanthemum inoculations, but no stem samples were taken. Development of bacterial blight symptoms in inoculated chrysanthemums was interpreted as good evidence that the inoculated bacterium was *E. chrysanthemi*. This pathogenicity test enabled us to determine which of the originally isolated bacteria were *E. chrysanthemi,* and to trace the extent of the penetration of the pathogen into the host.

The stem pieces fixed in FAA were dehydrated in a tertiary butyl alcohol series (10), infiltrated, and embedded in paraplast (Curtin-Matheson Scientific). Material to be sectioned was softened for 24 h in a solution of 90 ml of 1% sodium lauryl sulfate (Dreft) and 10 ml of glycerol (1) prior to sectioning at 10 μm on a rotary microtome. Transverse and longitudinal sections were mounted on chemically cleaned slides with Haupt's adhesive (10) and stained. Johansen's quadruple stain and Harris' hematoxylin and orange G were the staining schedules used in this study (10). The Harris' hematoxylin and orange G schedule was particularly useful for locating bacteria in the stem sections.

Histochemical tests (9, 14) for pectin (ruthenium red and the iron absorption method), lignin (Maule reaction), wound gum (phloroglucinol), cellulose (zinc-chlor-iodide, I-KI, and H_2SO_4), suberin (sudan IV), and starch (I-KI) were carried out on selected tissues. Sections were also studied under polarized light to detect the presence of crystalline cellulose and starch grains.

The vascular configuration of a chrysanthemum stem was determined by staining it with basic fuchsin. The plant was removed from soil and the root system washed with water. The root system was then placed in a solution of basic fuchsin (50 mg basic fuchsin, 2 ml 95% ethyl alcohol, 100 ml H_2O) for 24 h. Root tips were cut immediately after immersion in the staining solution. Following stain uptake, the plant was removed from the solution, washed with water and then ethyl alcohol, cut into sections containing several nodes, boiled for 6 min in a 0.5% KOH solution and dissected (10).

All sections were examined with a Leitz Ortholux Research microscopy and photographed on Kodak Plus-X Pan film by a Leitz Aristophot camera with a 10.16 × 12.70-cm (4 × 5-inch) Graflex back.

RESULTS.—*Isolations and inoculations.*—Isolation of bacteria from chrysanthemum stems began with the sample taken 12 h after inoculation with *E. chrysanthemi* and continued for all successive samples. Bacteria were isolated from the entire length of the stem (ca. 110 mm) in all plants sampled. Difficulty was encountered in separating *E. chrysanthemi* from the numerous unidentified bacteria also isolated from the stems. The unidentified bacteria generally grew faster in culture than *E. chrysanthemi,* and thus were more numerous in the nutrient broth. Although we isolated bacteria morphologically resembling *E. chrysanthemi* from all stem sections, pathogenicity tests indicated that we failed to isolate the pathogen consistently.

Approximately 51% of the bacteria, morphologically resembling *E. chrysanthemi,* rotted potato tuber tissue. Only 30% of the bacteria capable of rotting potato tubers were able to incite bacterial blight symptoms when inoculated into chrysanthemum plants. Although we did not recover the pathogen consistently, we feel confident that the bacteria we did recover which were able to rot potato tissue and cause bacterial blight symptoms in chrysanthemums were *E. chrysanthemi.*

Twelve h after inoculation, *E. chrysanthemi* was isolated 40 to 85 mm below the point of attachment of the inoculated leaf petiole in the three plants sampled. Seventy-four h following inoculation, *E. chrysanthemi* was isolated 80 to 100 mm down the stem in two of the plants sampled.

Histology of noninoculated plants.—Stems of the check chrysanthemum plants were examined to determine the healthy anatomy of a chrysanthemum stem at a stage of development comparable to that of the inoculated plants. Such information is essential when evaluating the effects of a pathogen on host tissue.

Near the apex, the healthy stem (Fig. 1) has an epidermis with multicellular T-shaped and glandular trichomes and slightly protruding stomata with flanged guard cells. The cortex is composed of numerous layers of radially isodiametric parenchyma cells and includes

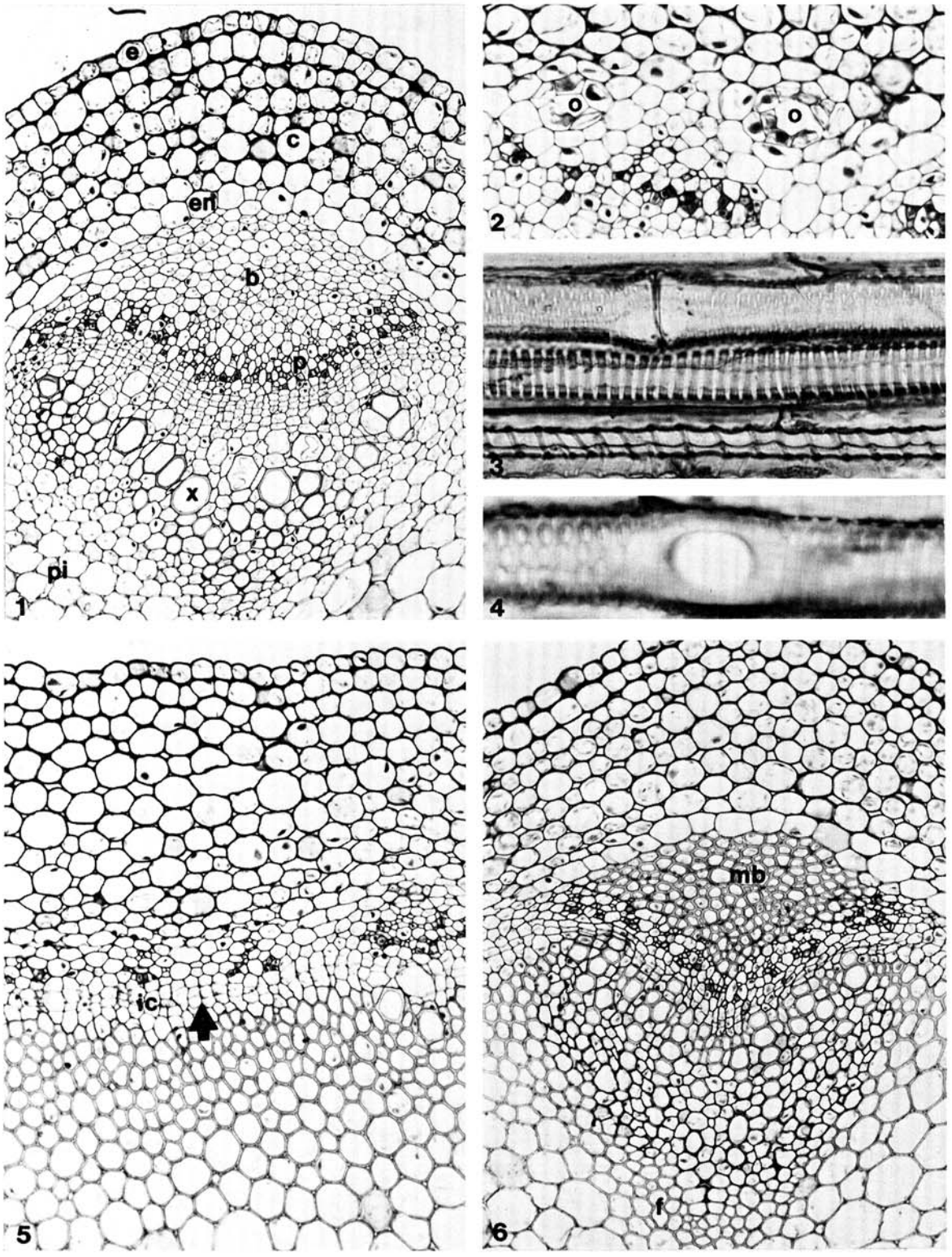

Fig. 1-6. Portions of sections of uninoculated stems of *Chrysanthemum morifolium*. **1)** Transverse section in which the epidermis (e), cortex (c), endodermoid layer (en), immature bundle cap (b), phloem (p), xylem (x), and pith (pi) may be seen (× 155). **2)** Transverse section in which oil ducts (o) are present at the interior edge of the cortex (× 315). **3)** Longitudinal section showing both helical and reticulate xylem vessel element thickenings (× 420). **4)** Longitudinal section showing a simple perforation plate of a xylem vessel element (× 720). **5)** Cross section through an older stem showing the interfasicular cambium (ic) and resultant secondary phloem. Note that the interfasicular cambium remains between the newly differentiated vascular bundle (arrow) (× 195). **6)** Transverse section through older stem tissue showing the mature bundle cap (mb) and the fiber layer (f) (× 165).

several schizigenous oil ducts adjacent to the endodermoid layer (Fig. 2) as described by Link (11) and Metcalfe and Chalk (12). The endodermoid layer consists of a single layer of thin-walled, rectangular parenchyma cells (Fig. 1) containing inclusions giving positive reactions for starch. Several vascular bundles composed of bundle caps of immature extraxylary fibers, the sieve tubes, companion cells and phloem fibers of the phloem and the xylem parenchyma, tracheids, and vessel elements of the xylem are located interior to the endodermoid layer. Xylem vessel elements have annular, helical, and reticulate secondary thickenings (Fig. 3) and simple perforation plates (Fig. 4). The vascular bundles are separated from each other by interfasicular parenchyma which becomes meristematic and produces additional vascular tissue as the plant matures (Fig. 5). Near the base of the stem, primary tissues begin to mature and each vascular bundle is bounded on the exterior side by a layer of mature extraxylary fibers (Fig. 6). A continuous fiber layer also forms between the pith and the vascular tissue (Fig. 6). The pith throughout the stem is composed of large radially isodiametric parenchyma cells.

Histology of inoculated plants.—As a result of the inoculation method used, *E. chrysanthemi* entered the main stem of chrysanthemum through the vascular system of the leaf trace. The bacteria moved through the xylem vessel elements of the petiole and entered the main stem at the point of attachment of the inoculated leaf. A few bacteria were occasionally observed in xylem vessel elements of the stem at a considerable distance from the inoculation point (Fig. 7, 19). In such cases, cells adjacent to the infected vessel elements frequently had notably thinner walls. Such reduced walls were faintly birefringent under polarized light.

Once in the main stem, *E. chrysanthemi* began to colonize the lumen of one or more xylem vessels of the vascular bundle from which the leaf trace diverged. On one occasion, the first noticeable reaction in the vascular tissue was slight hypertrophy of the xylem parenchyma and plugging of some xylem vessel elements (Fig. 8). Hypertrophy, when present, was followed by breakdown and disappearance of the xylem parenchyma cells (Fig. 9, 10). In some cases, what appeared to be cell wall fragments accumulated along the boundary between the vascular tissue and the pith tissue (Fig. 11, 12). At this point, the bacteria may be found in the cavities formed by disintegration of the xylem parenchyma as well as in the vessel elements (Fig. 13).

Following disintegration of the xylem parenchyma in the infected vascular bundle, the pith parenchyma cells frequently became separated from each other (Fig. 14) and eventually disintegrated (Fig. 20). After invasion of the pith cylinder, the bacterium apparently invaded other

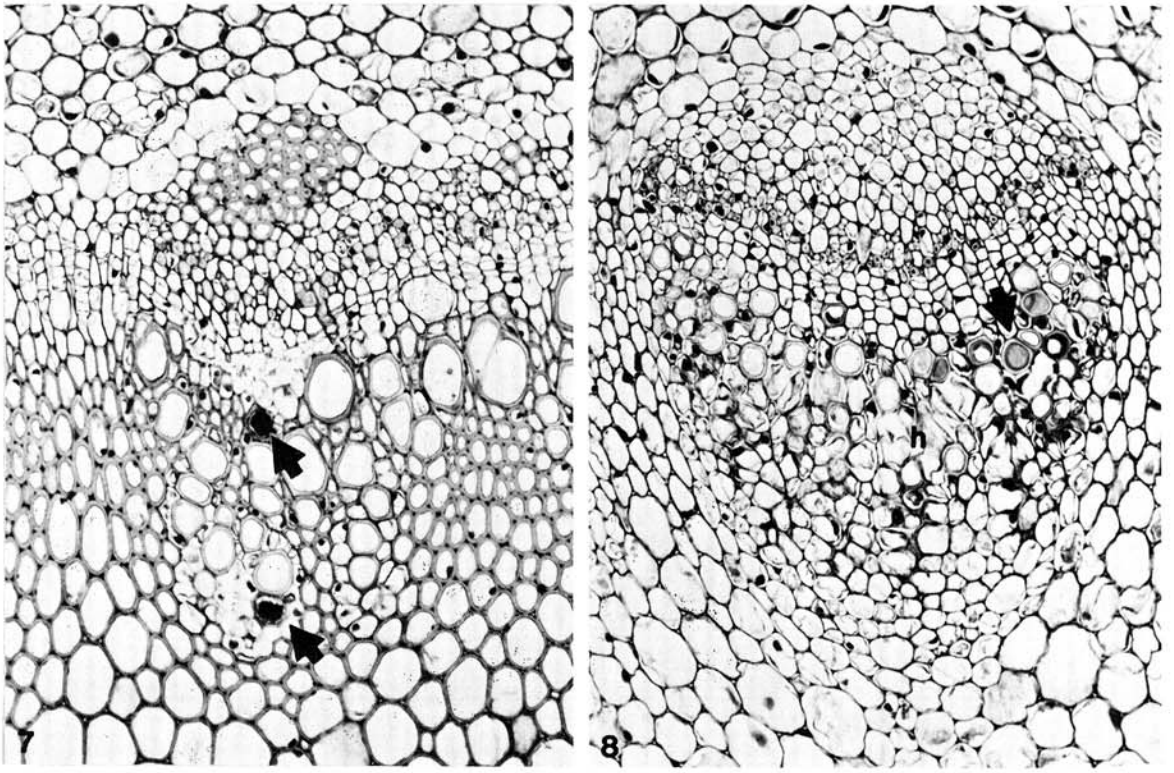

Fig. 7-8. Portions of transverse sections of *Chrysanthemum morifolium* stems inoculated with *Erwinia chrysanthemi*. **7)** Transverse section showing bacterial masses (arrows) in two xylem vessel elements. Note that the walls of the xylem parenchyma cells adjacent to the infected vessel elements are reduced in thickness (× 230). **8)** Transverse section showing xylem parenchyma hypertrophy (h) and several vascular plugs (arrow) (× 195).

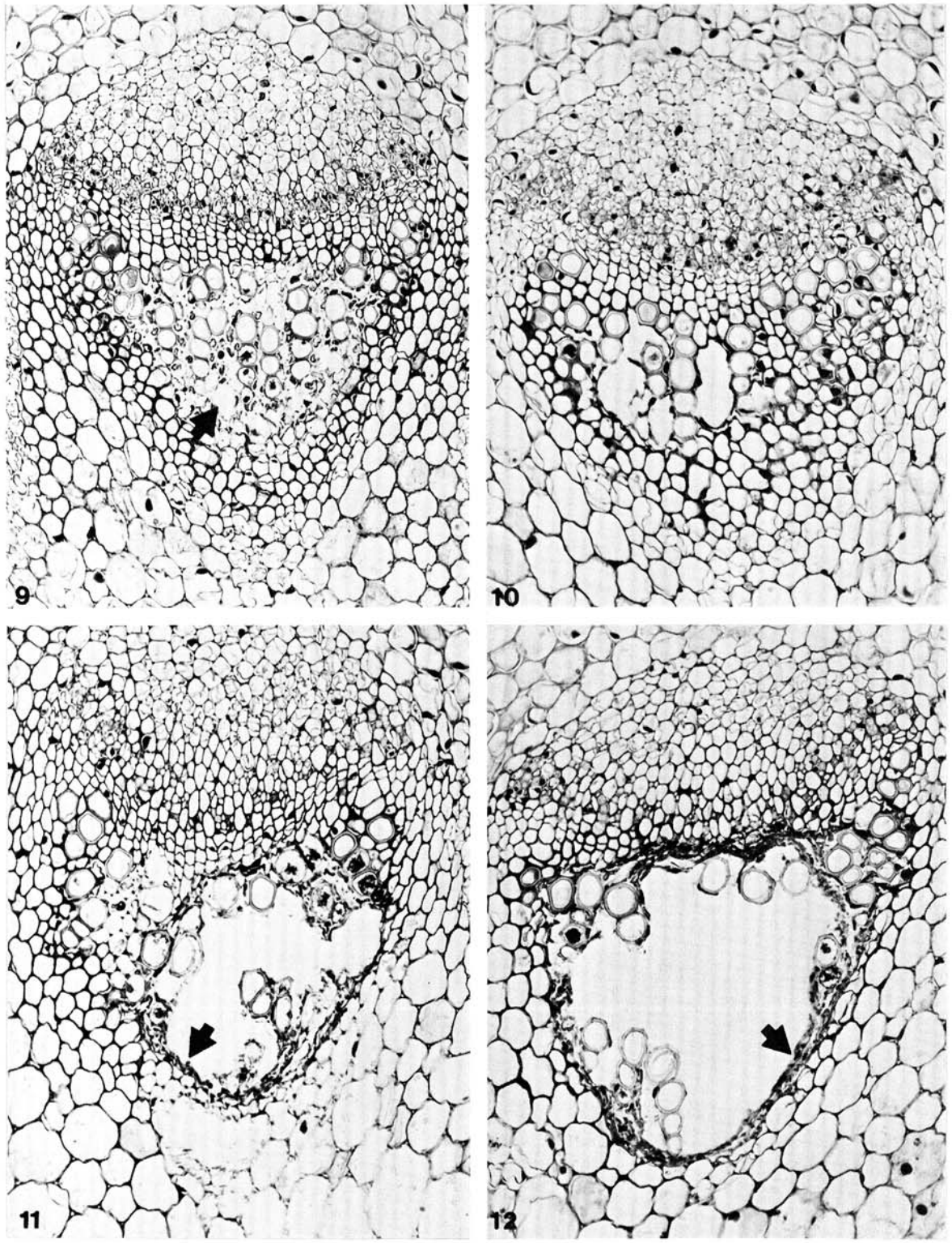

Fig. 9-12. Portions of transverse sections through stems of *Chrysanthemum morifolium* inoculated with *Erwinia chrysanthemi*, showing stages in the development of a vascular cavity. **9)** Early stage in vascular bundle disintegration in which some xylem parenchyma cells with cell walls reduced in thickness are present as well as cavities in which xylem cells are lacking (arrow) (× 175). **10)** A vascular cavity beginning to form as a result of xylem parenchyma disintegration. Note that no thin-walled xylem parenchyma cells are present (× 200). **11)** A later stage in vascular bundle disintegration in which unrecognizable tissue (arrow) is present at the cavity border (× 205). **12)** Enlarged vascular cavity and increased amounts of disintegrated host tissue (arrow) accumulated along the cavity boundary (× 210).

vascular bundles by separating and destroying the pith adjoining the bundle and then the xylem parenchyma of the bundle itself (Fig. 21). After invading another vascular bundle, *E. chrysanthemi* entered the xylem vessel elements, possibly through the pits and/or through gaps formed in the vessel element primary walls in response to the pathogen, and moved vertically in the stem (Fig. 22). Supporting evidence for vertical movement was seen in cross sections of stems taken above the jointure of the inoculated leaf. These sections contained several infected vascular bundles, but the pith remained intact. The pith was destroyed for several millimeters below the leaf jointure. *Erwinia chrysanthemi* must have moved vertically in the vascular elements rather than through the pith to have caused such an anatomical manifestation.

As development of the pathogen in the stem progressed, the fasicular cambium in many bundles became compressed, vessel elements became plugged, and tissue maceration may extend into the phloem. As breakdown of xylem parenchyma continued, large masses of bacteria developed in the resultant cavaties, and eventually in the area where the pith disintegrated. Occasionally, unaffected vascular bundles may adjoin disintegrating bundles, possibly indicating that the bacteria moved from bundle to bundle through the pith, rather than through the interfasicular parenchyma. Further evidence for such pathogen movement is provided by the presence of narrow channels or spaces (Fig. 15) between cells of the infected pith and protoxylem cells of vascular bundles in the early stages of cell breakdown. In one case bacteria were found in a channel connecting the disintegrated pith and an apparently unaffected vascular bundle. In many cases, the narrow channels present during the early stages of horizontal pathogen spread through the stem become greatly enlarged (Fig. 16). At this point, the vascular tissue of the bundle was completely exposed to the pith cavity formed by cell disintegration (Fig. 21, 22).

As colonization and cell disintegration encompass more tissue, both horizontally and vertically in the stem, a large stem crack may occur and expose the disintegrated pith area to the stem exterior. Vessel elements and occasionally large fragments of intact vascular tissue become disassociated from the fasicular cambium and were found in the pith region (Fig. 17). Isolated helical secondary thickenings from xylem vessel elements were frequently present in the pith cavity, as were large masses of bacteria (Fig. 18). Observations of infected stems up to 96 h after inoculation indicated that the stem crack symptom and the hollow stem were confined to the 35 to 40 mm directly below the inoculated leaf. Below this area, the destruction was reduced, frequently confined to the vascular bundles, and consisted of xylem parenchyma maceration.

Throughout the study, we noted that tissue separation and cell disintegration were inconsistent. In some cases, cells of the pith and xylem parenchyma were destroyed,

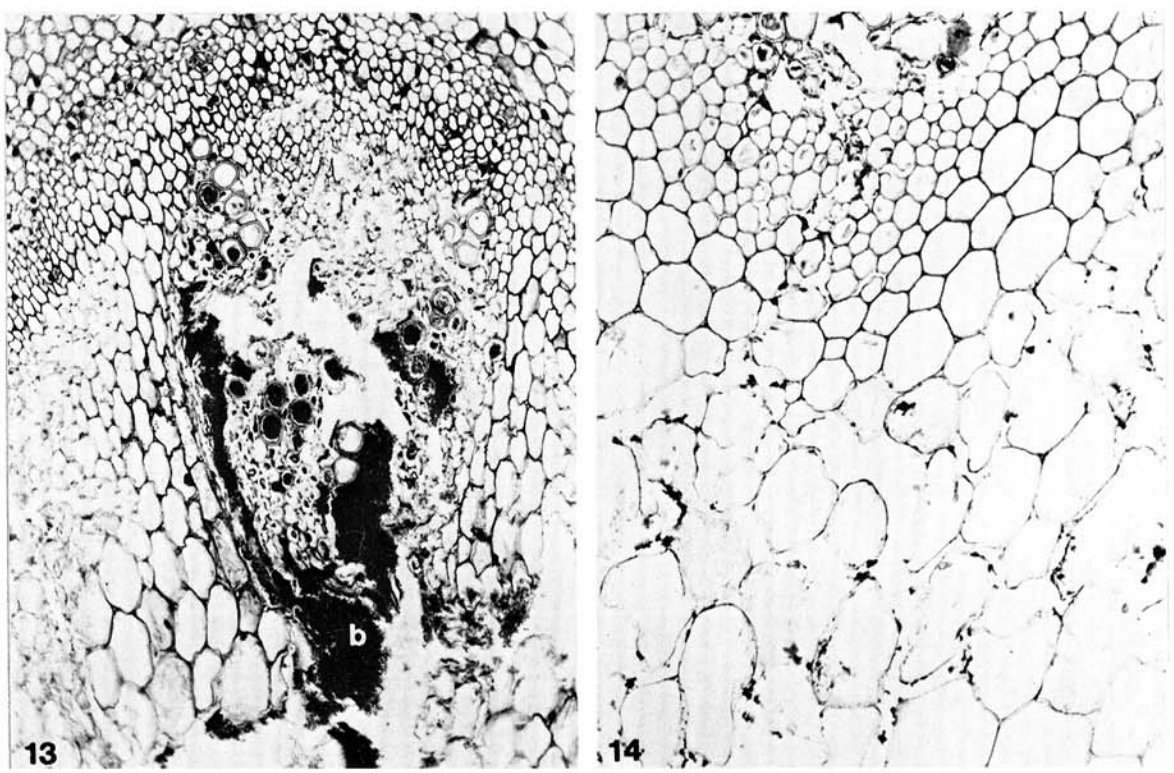

Fig. 13-14. Portions of transverse sections of *Chrysanthemum morifolium* stems inoculated with *Erwinia chrysanthemi*. **13)** Advanced stage in vascular cavity formation in which bacterial masses (b) are present both inside the xylem vessel elements and in the cavity itself (× 155). **14)** Pith cells in an early stage of disintegration in which they are entire, but separated from each other (× 190).

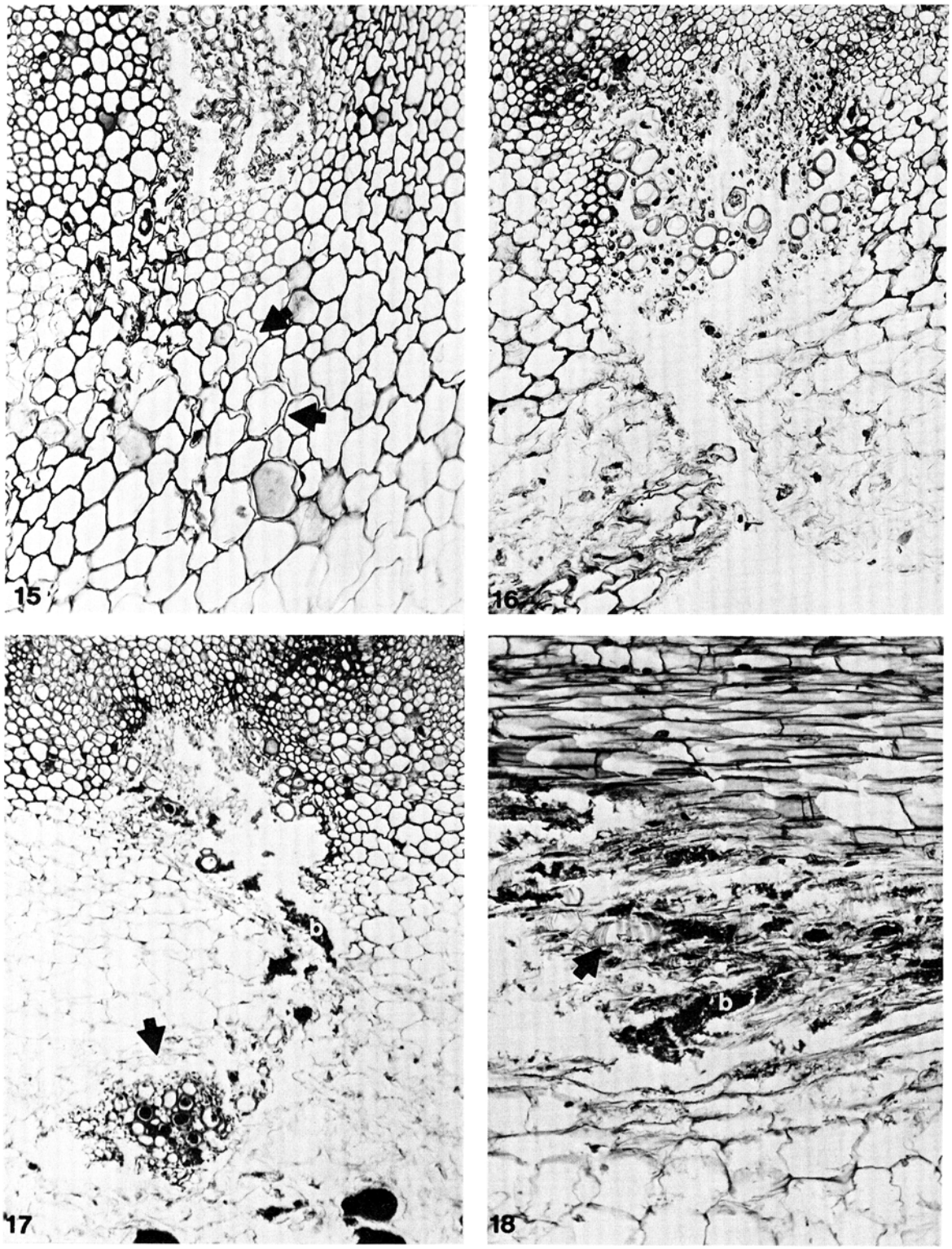

Fig. 15-18. Portions of sections of *Chrysanthemum morifolium* stems infected with *Erwinia chrysanthemi* showing stages in channel formation and vascular disruption. Fig. 15-17 are transverse sections while Fig. 18 is a longitudinal section. **15)** Small, narrow "channels" or gaps (arrows) are present between the disintegrating vascular bundle and the macerated pith (× 150). **16)** Advanced stage in "channel" formation in which the channel has increased in width, opening the disintegrating bundle directly into the disintegrating pith (× 150). **17)** Note the large amount of xylem tissue disintegration and the presence of the tissue from the vascular bundle in the pith tissue (arrow). Bacterial masses (b) are present in the "channel" between the vascular tissue in the pith and the remaining portion of the vascular bundle (× 100). **18)** Longitudinal section showing detached helical secondary xylem vessel element thickenings (arrow), total disorganization of the vascular system and bacterial masses (b) throughout the disintegrating tissue (× 175).

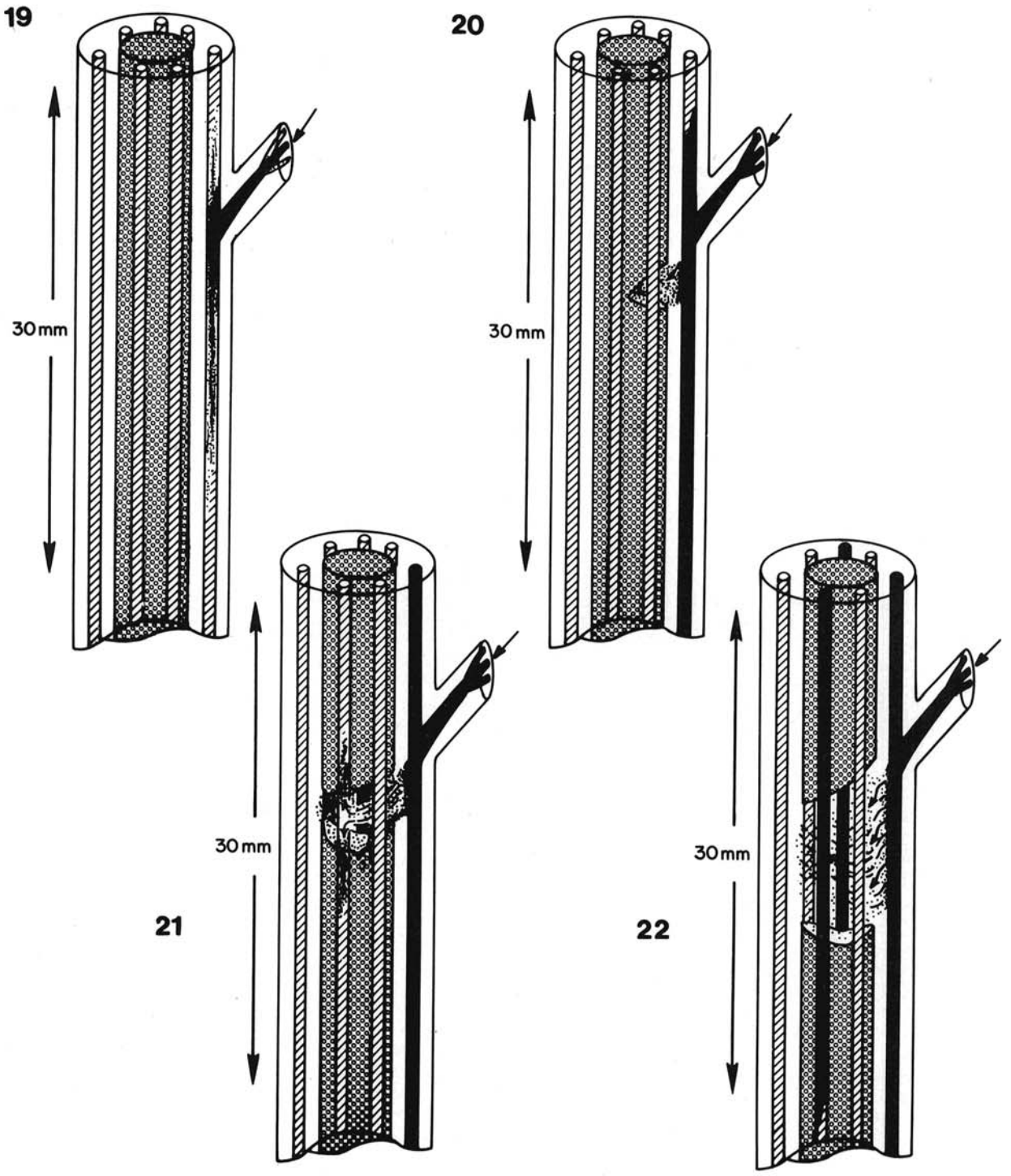

Fig. 19-22. Diagrams illustrating the stages in the development of *Erwinia chrysanthemi* and tissue disintegration in stems of *Chrysanthemum morifolium* following inoculation of a young leaf midrib with the bacterium. Times noted are approximate. **19)** Eight hours after inoculation, bacteria (black shading) entered the chrysanthemum stem through the midrib of the third youngest leaf (arrow) and colonized the vascular bundle from which the leaf trace emerged. **20)** After 32 h, the pathogen colonized the vascular bundle for a distance of 47 mm below the point of attachment of the inoculated leaf trace. In addition, *E. chrysanthemi* broke out of the vascular bundle (arrow) and began to destroy the pith tissue. **21)** Pith colonization and disintegration was extensive after 48 h and gave the bacteria access to additional vascular bundles which they penetrated (arrows). **22)** Once in the xylem vessel elements of the additional vascular bundles, usually within 56 h, the bacteria moved vertically in the stem. Pith disintegration also advanced vertically, but at a much slower rate.

while in other areas of the same section cells were merely separated from adjoining cells. When xylem parenchyma cells were separated and not disintegrated, the cell walls were always considerably reduced in thickness. The thin-walled cells, however, were still faintly birefringent under polarized light.

Histochemical tests for pectic substances generally showed a slight reduction in the color intensity of the reaction in areas of cell disintegration and in several instances, the iron absorption reaction yielded blue-green color in the unrecognizable tissue accumulated at the vascular bundle boundaries. The significance of the blue-green color was not determined. The material surrounding the bacterial masses gave the typical positive reaction for pectic substances as did the vascular plugs.

The zinc-chlor-iodide test for cellulose yielded positive results in all tissues examined. Observations under polarized light revealed strong birefringence in all cells with two exceptions. The thin-walled xylem parenchyma cells frequently present in disintegrating vascular bundles were faintly birefringent as was the collapsed tissue at the borders of cavities in the pith and vascular tissue. The reduction in the intensity of birefringence in these two cases indicates that some cellulolytic material was removed or changed in the walls of those cells. Thus, there is some evidence of cellulolytic enzyme activity although such activity does not appear to be extensive.

Histochemical tests for starch, suberin, and wound gum revealed starch grains restricted to the endodermoid layer, suberin confined to the stem cuticle, and no wound gum in the vascular plugs. Results from the Maule reaction for lignin were inconclusive.

The bacterial blight organism, *E. chrysanthemi,* caused no changes in the epidermis, cortex, or endodermoid layer of the stem, with the exception of tissue separation caused by stem cracks. The pathogen caused separation of cells and loss of identity of tissues in the vascular bundles and the pith and eventually only remnants of the components of the vascular bundles and pith remained which we call the hollow area of the infected stem.

DISCUSSION.—The mechanism by which *E. chrysanthemi* enters healthy chrysanthemum plants is not clear. It has been suggested that the pathogen enters the plant through the wounds caused when the plants are pinched or when cuttings are taken (5). This would provide an opportunity for the bacterial cells to increase initially in the pith tissue and to enter the vascular system, thus appearing to develop and to move through the vascular system. To eliminate this possibility, the pathogen was introduced directly into the vascular tissue of the leaf midvein, thus insuring that the bacteria entered the main stem through the vascular tissue of the leaf trace. In all cases, observations made during early disease development revealed that mainstem infection apparently was confined to a single vascular bundle. Although we did not observe the leaf trace from the inoculated leaf, at later stages in the disease development, we did observe bacteria in the leaf traces of other leaves. We concluded that our method of inoculation resulted in the initial development of the pathogen in the vascular tissue of the main stem. The occurrence of bacteria in xylem vessel elements of the infected bundle, several centimeters below the infected leaf trace, was direct evidence of the ability of *E. chrysanthemi* to develop and to become distributed through the xylem vessel elements of the chrysanthemum.

Contrary to the Burkholder et al. (5) description of brown streaks in the cortex of infected plants, we failed to observe any such streaks in cortical tissue and further, found no evidence of any involvement of the cortex in the disease syndrome. We do concur with their observations of brown vascular bundles although the present study did not reveal the substances which caused such browning.

In most inoculated plants, we observed greater host cell destruction and pith disintegration a few millimeters below the attachment of the inoculated leaf to the main stem. In advanced stages, the stem was hollow in this area. Stem tissue destruction and hollowness in this area probably reflect the joining of the inoculated leaf trace with the vascular system of the main stem. Thus, bacteria were present in this region longer than in the rest of the stem and consequently, caused greater cell destruction. In addition, observations show that the pathogen moved down the vessel elements more freely than up. It is quite possible that pith disintegration might have become more extensive in the vertical direction had the plants been kept in favorable conditions for more than 96 h.

Additional evidence of the blight organism's preference for vascular tissue was seen in the spread of the bacterium through the pith tissue and into additional vascular bundles (Fig. 19, 20, 21, 22). Such spread took place below the region in which the pathogen entered the stem. Later observations of the stem above the inoculated leaf trace showed many infected, disintegrating vascular bundles, but intact pith tissue. The only way the pathogen could have appeared in those bundles, above the inoculation point, was to move through the vascular system, indicating that *E. chrysanthemi* is systemic in the chrysanthemum.

We failed to isolate the pathogen consistently throughout the chrysanthemum stem. Since we observed histological evidence of the pathogen's presence in areas from which *E. chrysanthemi* was not isolated, our isolation technique was not 100% efficient for *E. chrysanthemi.* The presence of large numbers of apparently saprophytic bacteria in the chrysanthemum stem greatly compounded the problem of isolating the pathogen. Such a resident bacterial population has been reported in carnation (7) and Burkholder (4) reported the presence of nonpathogenic bacteria in lesions in plants. Our studies did not determine whether the saprophytic bacteria were involved in the bacterial blight syndrome as was the case with *Pseudomonas caryophylli* and *Corynebacterium* sp. in carnation (3). It is interesting to note that many of the bacteria in chrysanthemum, while not *E. chrysanthemi,* were capable of causing soft rot in potato tubers, but were unable to produce symptoms when inoculated into chrysanthemum plants.

Garibaldi and Bateman (8) isolated several polygalacturonic acid *trans*-eliminases produced by carnation and philodendron isolates of *E. chrysanthemi,* and found them capable of degrading pectic acid and of macerating various tuber tissues. They noted a difference in the ability of various enzyme fractions to degrade pectic substances from different plants.

The various histochemical tests for pectic substances used in this study failed to produce any conclusive

evidence concerning the reduction of pectic substances in the host tissue. Such a reduction was expected due to the massive tissue disintegration observed; however, even in unrecognizable tissue we obtained a color reaction which was interpreted as a positive test. As Bateman and Millar (2) mentioned, histochemical tests are not always reliable. It is possible to get a positive reaction for pectic substances even though they have been partially degraded. Despite histochemical evidence to the contrary, our observations of separated pith cells and vascular fragments seem to indicate the destruction or weakening of the middle lamella.

Studies with polarized light gave some evidence of cellulose reduction in the xylem parenchyma cells with reduced wall thickness. Thus, we feel that cellulases may play a role in bacterial blight of chrysanthemum.

The wilt symptom that is part of the blight syndrome appears to be a result of the disarrangement of the xylem vessel elements, due in part to the removal by disintegration of the supporting xylem parenchyma cells. Such physical discontinuity probably breaks the transpiration stream and water no longer moves up the stem. Some vascular plugging with substances positive for pectin as well as with bacterial masses was noted, but did not appear to be the primary cause of wilting.

LITERATURE CITED

1. ALCORN, S. M., and P. A. ARK. 1953. Softening paraffin-embedded plant tissues. Stain Technol. 28:55-56.
2. BATEMAN, D. F., and R. L. MILLAR. 1966. Pectic enzymes in tissue degradation. Annu. Rev. Phytopathol. 4:119-146.
3. BRATHWAITE, C. W. D., and R. S. DICKEY. 1971. Role of cellular permeability alterations and pectic and cellulolytic enzymes in the maceration of carnation tissue by Pseudomonas caryophylli and Corynebacterium sp. Phytopathology 61:476-483.
4. BURKHOLDER, W. H. 1948. Bacteria as plant pathogens. Annu. Rev. Microbiol. 2:389-412.
5. BURKHOLDER, W. H., L. A. MC FADDEN, and A. W. DIMOCK. 1953. A bacterial blight of chrysanthemums. Phytopathology 43:522-526.
6. CREELMAN, D. W. 1966. Summary of the prevalence of plant diseases in Canada in 1965. Can. Plant Dis. Surv. 46:37-79.
7. DICKEY, R. S., and P. E. NELSON. 1970. Pseudomonas caryophylli in carnation. IV. Unidentified bacteria isolated from carnation. Phytopathology 60:647-653.
8. GARIBALDI, A., and D. F. BATEMAN. 1971. Pectic enzymes produced by Erwinia chrysanthemi and their effects on plant tissue. Physiol. Plant Pathol. 1:25-40.
9. JENSEN, W. A. 1962. Botanical histochemistry. W. H. Freeman and Co., San Francisco. 408 p.
10. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., New York. 523 p.
11. LINK, C. B. 1940. An anatomical study of the seedling of Chrysanthemum morifolium Bailey, Ph.D. Thesis, Ohio State University, Columbus. 42 p.
12. METCALFE, C. R., and L. CHALK. 1950. Anatomy of the dicotyledons; leaves, stem, and wood in relation to taxonomy, with notes on economic uses. Clarendon Press, Oxford, England. 1,500 p.
13. RAWLINS, T. E. 1933. Phytopathological and botanical research methods. John Wiley and Sons, New York. 156 p.
14. RAWLINS, T. E., and W. N. TAKAHASHI. 1952. Technics of plant histochemistry and virology. The National Press, Millbrae, Calif. 125 p.