A Histological Comparison of the Response of a Chrysanthemum Cultivar Susceptible to *Erwinia chrysanthemi* and *E. carotovora* subsp. *carotovora*

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Contribution 1151, Department of Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication 4 February 1980 as Journal Series Paper 5908.

Accepted for publication 20 June 1980.

ABSTRACT

Pennypacker, B. W., Dickey, R. S., and Nelson, P. E. 1981. A histological comparison of the response of a chrysanthemum cultivar susceptible to *Erwinia chrysanthemi* and *E. carotovora* subsp. *carotovora*. Phytopathology 71:138-140.

A comparison was made between the response of *Chrysanthemum morifolium* 'Giant #4 Indianapolis White' to *Erwinia carotovora* subsp. *carotovora* and its response to *E. chrysanthemi*. Both of these organisms caused bacterial blight symptoms in chrysanthemum under favorable conditions. Histological studies revealed that these pathogens caused almost identical anatomical changes in the host plant. Vascular bundle maceration followed by extensive pith cell breakdown occurred in plants inoculated with either of these *Erwinia* species. Histochemical tests indicated that enzymatic degradation of the middle lamella was probably the primary mechanism responsible for cell breakdown and plant collapse in both cases.

Erwinia chrysanthemi, the causal organism of bacterial blight of *Chrysanthemum morifolium* Ramat. (1) and *E. carotovora* subsp. *carotovora* (*E. c.* subsp. *carotovora*) are commonly isolated from chrysanthemum plants showing symptoms of bacterial blight (2). The soft rot symptoms induced in chrysanthemum by these two pathogens under conditions of high humidity and high temperature are similar. In view of this symptom similarity, we conducted a descriptive histological study to determine whether the anatomical response of the host to infection by *E. c.* subsp. *carotovora* differed in any way from the response previously reported for *E. chrysanthemi* (4).

MATERIALS AND METHODS

Rooted cuttings of chrysanthemum cultivar Giant #4 Indianapolis White (supplied by Yoder Brothers, Barberton, OH 44203) were potted in 10.2-cm-diameter pots of a steam-treated peat:perlite:soil (1:1:1, v/v) mixture and grown under incandescent light for 3 wk prior to inoculation. Twenty-six plants were inoculated with *E. c.* subsp. *carotovora* (strain 311 from chrysanthemum) and 13 plants were inoculated with *E. chrysanthemi* (strain EC-16 from chrysanthemum). Five plants were used as uninoculated check plants. Inoculum was prepared and petiole inoculations were carried out as described previously (4). The experiment was designed so that two plants inoculated with *E. c.* subsp. *carotovora* and one plant inoculated with *E. chrysanthemi* would be sampled at 2, 4, 6, 8, 10, 12, 17, 24, 31, 48, 55, 72, 96 hr after inoculation. One uninoculated control plant was sampled every 24 hr. Plants infected with *E. c.* subsp. *carotovora* exhibited the terminal symptom of total stem collapse 48 hr after inoculation, at which time the experiment was terminated.

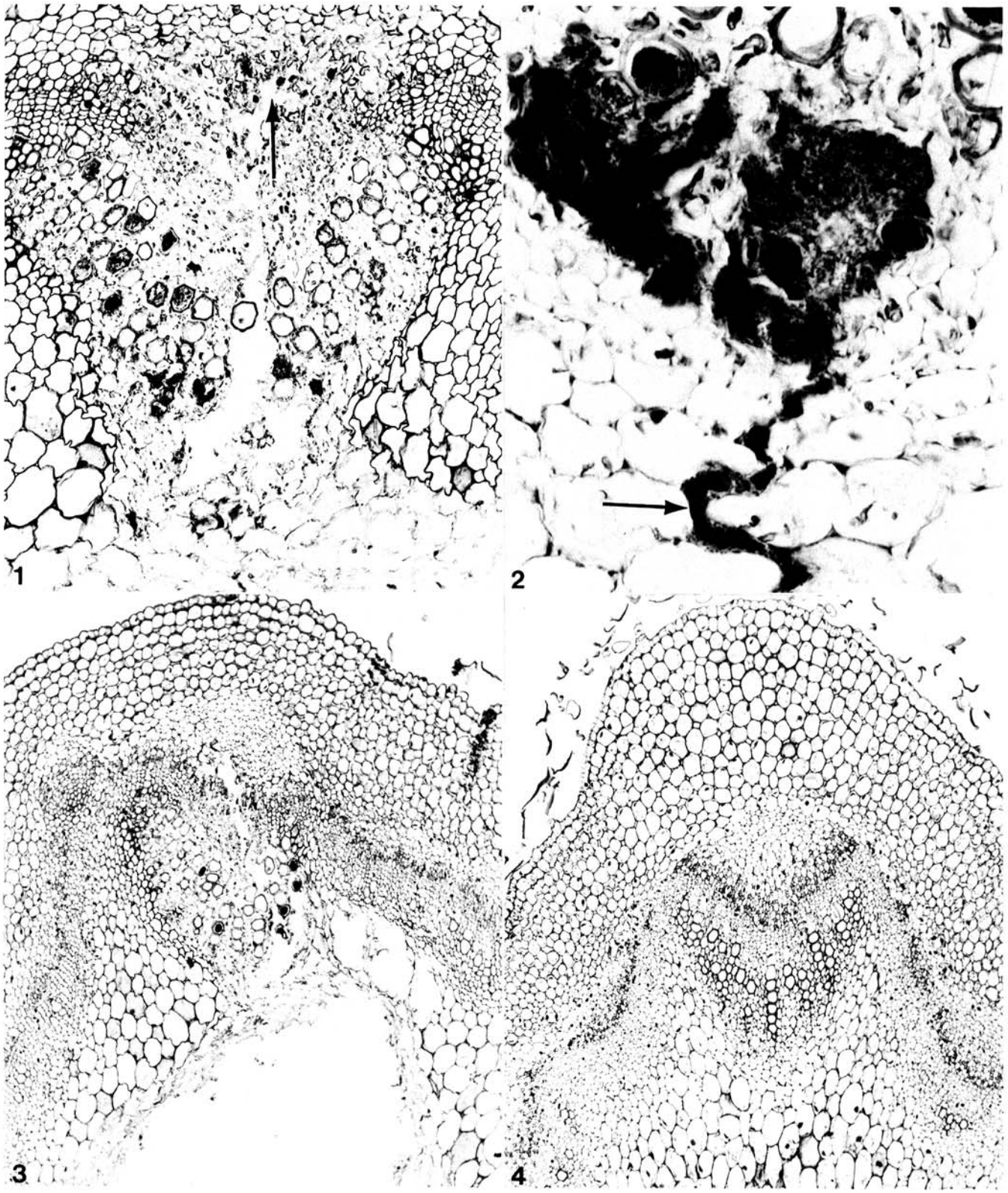
Bacterial isolation and identification and histological sampling were carried out as described previously (4). The histological specimens were dehydrated in a tertiary butyl alcohol series (3) and embedded in Paraplast (Scientific Products, Obetz, OH 43207). Transverse and longitudinal serial sections were cut (at 10 μm) on a Leitz rotary microtome and were mounted on chemically cleaned slides with Haupt's adhesive (3). The histological sections were stained with Harris' Hematoxylin and Orange G (3) and were examined under a Leitz Ortholux microscope.

Selected sections were examined under polarized light for the presence of cellulose. Other sections were tested for the presence of pectin with the iron absorption test (5).

0031-949X/81/02013803/$03.00/0

RESULTS

Bacteria were isolated from all plants sampled in this study. *E. c.* subsp. *carotovora* was isolated from the stem 35 mm from the inoculation point 2 hr after inoculation and 85 mm from the inoculation point 4 hr after inoculation. *E. chrysanthemi* was not isolated from the stem 2 hr after inoculation, but was recovered 100 mm from the inoculation point 4 hr after inoculation. Identification of the isolated bacterial colonies was based on the ability of the isolates to rot potato tuber tissue and to cause typical



Figs. 1–4. Portions of cross sections of stems of *Chrysanthemum morifolium* 'Giant #4 Indianapolis White.' Stems in Figs. 1–3 were inoculated with *Erwinia carotovora* subsp. *carotovora* (strain 311) and the stem in Fig. 4 was inoculated with *E. chrysanthemi* (strain EC-16). **1,** 31 hr after inoculation with *E. c.* subsp. *carotovora*. Note that tissue maceration has occurred in the pith, xylem parenchyma, vascular cambium, phloem, and fibers (arrow) (×165). **2,** Bacterial masses present in the xylem vessel elements and in the vascular cavity formed by maceration of the xylem parenchyma cells. Note that the bacteria are intercellular in the pith (arrow) (×450). **3,** 24 hr after inoculation with *E. c.* subsp. *carotovora*, several vascular bundles as well as a portion of the pith have been destroyed (×85). **4,** 24 hr after inoculation with *E. chrysanthemi* no histological changes are apparent (×85).

symptoms of bacterial blight in inoculated chrysanthemum plants. Bacteria rotting both types of tissue were considered to be either *E. c.* subsp. *carotovora* or *E. chrysanthemi*.

Histology of plants inoculated with *E. carotovora* subsp. *carotovora*. *E. c.* subsp. *carotovora* was introduced into the chrysanthemum stem via a leaf trace. Consequently, the first stem tissue the pathogen encountered was the vascular bundle associated with the leaf trace. This inoculation method corresponded to the method previously reported for *E. chrysanthemi* in Giant #4 Indianapolis White (4). Seventeen hours after inoculation, *E. c.* subsp. *carotovora* caused extensive anatomical changes in the host. The first evidence of pathogen activity was the total maceration of one vascular bundle including the xylem parenchyma cells, vascular cambium, phloem, and fiber cap. Pith tissue adjacent to the disintegrated vascular bundle was in the early stages of cell separation and maceration.

As the time from inoculation increased, so did the extent of host tissue destruction. Between 24 and 31 hr after infection, *E. c.* subsp. *carotovora* moved out of the initially infected vascular bundle and caused extensive pith cell maceration. In addition, the pathogen invaded several other vascular bundles, apparently via the pith, and destroyed them in a centrifugal direction (Fig. 1).

E. c. subsp. *carotovora* was present in some of the xylem vessel elements in infected vascular bundles as well as in the pith tissue and in the pith and vascular cavities. The bacteria were found to be intercellular in the pith (Fig. 2) although they were present both intercellularly and intracellularly in the vascular bundles.

Forty-eight hours after inoculation, the stem tissue had cracked open revealing extensive internal discoloration and tissue destruction. *E. c.* subsp. *carotovora* completely destroyed the pith tissue of the stem for a vertical distance of several centimeters. All vascular bundles in the stem were in various stages of maceration and in several cases fragments of the vascular bundle were separated from their point of origin. Near the edge of the stem crack, some fiber cells appeared to be crushed while others were macerated. The crushing was observed only in the stem area directly adjacent to the stem crack. All other tissue destruction appeared to be due to maceration.

Examination of tissue under polarized light revealed a slight reduction in the birefringence of some xylem vessel elements in the disintegrating vascular bundles and in some pith cells. The iron absorption test for pectic substances revealed that the amount of pectic materials was reduced in the middle lamella of the xylem parenchyma cells, the vascular cambium, phloem, and fiber cells, and in the pith cells when maceration was evident in these tissues. There was evidence that the amount of pectic material was reduced in the cortical and epidermal cells adjacent to the stem crack although no change in the amount of pectic material was evident in all other cortical and epidermal cells.

Histology of plants inoculated with *E. chrysanthemi*. Forty-eight hours after inoculation, *E. chrysanthemi* caused maceration of the xylem parenchyma cells of several vascular bundles as well as the pith. The cell destruction caused by *E. chrysanthemi* was identical to that reported previously (4) and also to that caused by *E. c.* subsp. *carotovora*.

The major difference between the pathogenicities of these two pathogens in chrysanthemum cultivar Giant #4 Indianapolis White appears to be in the rate at which they cause host cell destruction (Figs. 3 and 4). In these studies, *E. c.* subsp. *carotovora* completely macerated a vascular bundle and some pith tissue 31 hr earlier than did *E. chrysanthemi*.

DISCUSSION

Results of this study showed that the isolates of *E. chrysanthemi* and *E. c.* subsp. *carotovora* caused similar reactions in the chrysanthemum cultivar Giant #4 Indianapolis White. Both pathogens destroyed the initially infected vascular bundle and then invaded the pith tissue causing extensive maceration. Movement through the pith tissue by both *Erwinia* species apparently was independent of vertical movement through the xylem vessel elements.

Extensive maceration of both the pith and the vascular tissue of cultivar Giant #4 Indianapolis White infected by *E. c.* subsp. *carotovora* often resulted in stem cracking. Anatomical and histochemical evidence indicated that the crack may arise from a combination of two different mechanisms. The pectin test revealed a reduction in pectic material in the middle lamella of the cortical and epidermal cells on either side of the stem crack. In addition, fiber cells along the edges of the crack appeared to be crushed. Therefore, stem cracking as caused by this isolate of *E. c.* subsp. *carotovora* might be a combination of enzymatic degradation and cell collapse due to pressure.

Damage to the host tissue caused by *E. c.* subsp. *carotovora* and *E. chrysanthemi* was similar and both pathogens were present in the chrysanthemum stems within 4 hr after inoculation. Although *E. c.* subsp. *carotovora* caused a more rapid maceration and cell disruption in inoculated plants, this may only reflect a difference in the virulence of the two strains used in this experiment. The inoculation method used did not allow for the same number of bacterial cells to be inserted into the leaf petiole each time a plant was inoculated and this factor also may affect the rate of maceration and cell destruction in inoculated plants.

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