Pathological Anatomy of Olive Tumors Caused by *Pseudomonas savastanoi*

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ABSTRACT

Samples from healthy and infected olive, *Olea europaea* L., twigs with *Pseudomonas savastanoi* were sectioned and studied before and after fixation. The following anatomical characteristics of tumor formation in olive were observed: (i) hypertrophy and hyperplasia; (ii) dissolution and disruption of cells resulting in fissures around the bacteria; (iii) differentiation of tumor cells into disarranged and malformed tracheary elements; and (iv) bacteria overwinter in pockets within the xylem and under favorable conditions will move through fissures toward outside the tumor.

INTRODUCTION

Olive knot, a bacterial disease of a very common occurrence on olive trees in the Libyan Arab Republic, is induced by *Pseudomonas savastanoi* (E. F. Sm.) Stevens (2). The disease affects twigs, branches, trunks, roots, leaves, fruit pedicels and sometimes the fruit itself (13,14). Irregular, spongy, more or less hard, knotty galls start as small swellings and increase in size to several inches with irregular fissures (Fig. 1, 2). Terminal shoots are dwarfed and severely affected twigs first lose their foliage and ultimately die; whole tree might be killed.

This paper reports the findings relative to the localization of the bacteria in the host tissue and illustrates a possible mechanism for tumor formation.

MATERIALS AND METHODS

Healthy and diseased (Fig. 1, 2) twig samples of corresponding ages and degrees of maturity were collected from field-grown olive trees at Zaweit El-Dahman, Tripoli. Specimens were studied after being fixed in formalin — acetic acid — alcohol method, embedded in paraffin, and sectioned on a rotary microtome (4,5). The tissue sections were stained with safranin — fast green (4) and mounted in Canada balsam.

Nucleoli, chromosomes, cuticle and lignified cell walls stain red; the remaining structures stain green.

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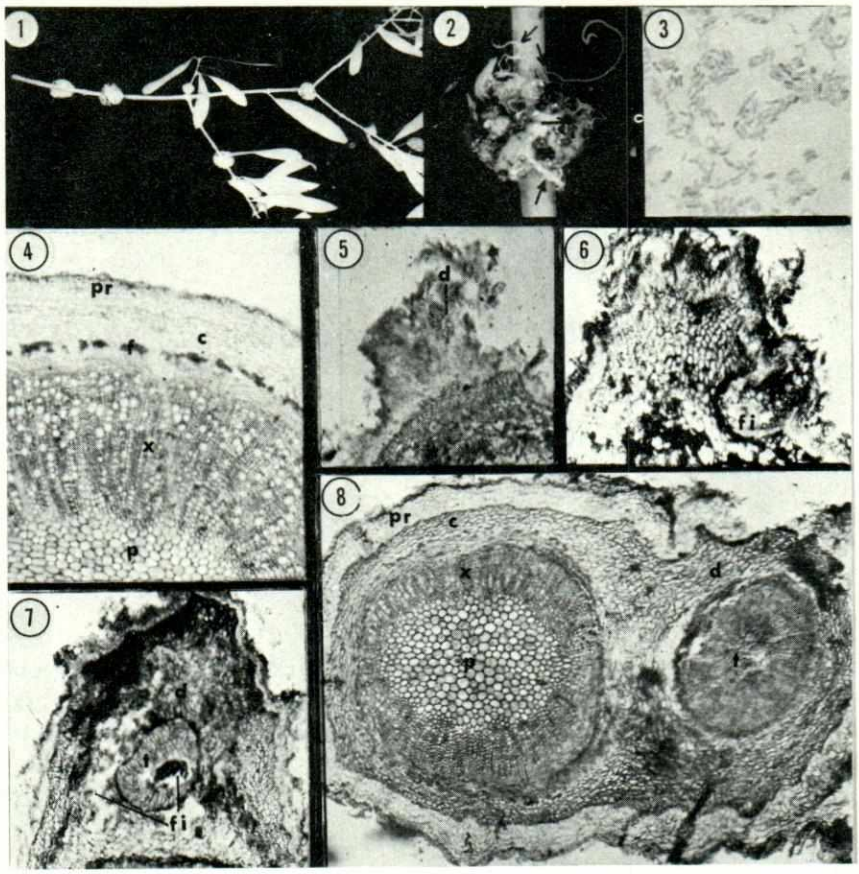
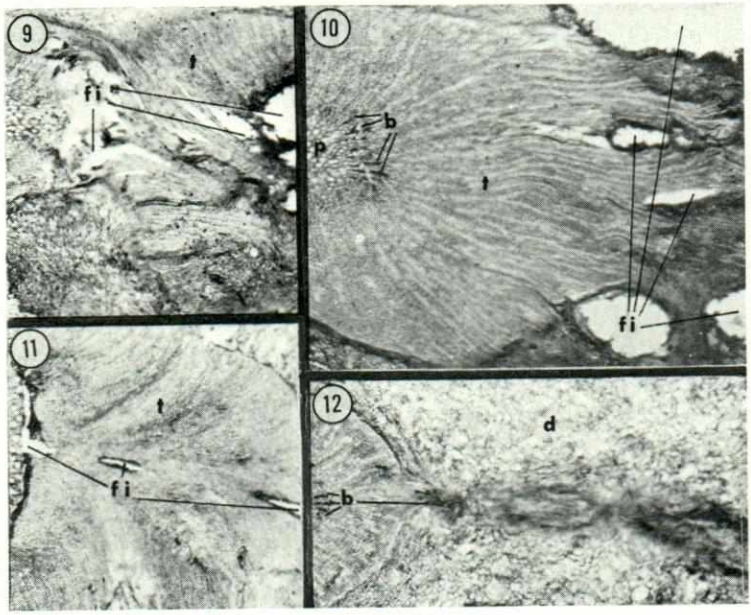


Figs. 1–8.



Figs. 9–12.

RESULTS AND DISCUSSION

Figure 4 is a cross section in olive twigs employed in this study. It shows periderm, several rows of the cortical parenchyma, fiber bundles of thick walled sclerenchyma, phloem, cambium and xylem cylinder. Xylem tissue is usually formed from rows of tracheary elements separated by one or more rows of xylem parenchyma. Lumen is filled with parenchyma pith cells.

Bacteria enter the host through wounds, often leaf scars frost injury (3,13). The first step of tumor formation is abnormal enlargment and cell division of the host tissue at the infection site (Fig. 5, 6). This might occur in the periderm or cortex depending on the depth of the wound and pathogen penetration into the host tissues.

Bacterial cavities or fissures (Fig. 6, 7) could be formed by the dissolution and disruption of host cells which are affected by the presence of the pathogen, while intact cells around the cavity will be hypertrophied causing pressure upon normal cells beyond them (Fig. 6). This suggests that bacterial enzymes might be involved in the disintegration of host cells. Magie (7) reported that *P. savastanoi* is able to produce several enzymes such as pectinase, xylanase, cellulase, and cellobiase *in vitro*.

A more advanced stage of hyperplasia is shown in Fig. 7. The cortical cells have undergone numerous divisions, whereas those around the fissures have been differentiated into disarranged and malformed tracheary elements. The hyperplastic cell appeared more dense in the cytoplasm and often the nucleolus of the nucleus was found to be larger than the nucleolus in comparable normal cells.

Figure 8 shows a cross section through an infected twig in which tumor development has been advanced. Sclerenchyma and phloem tissues cannot be distinguished outside the cambium; only hyperplastic tissue. Infection might reach the xylem tissue through its parenchyma (Fig. 9). It appears that all undifferentiated tumor cells have been transformed into tracheary elements (Fig. 9, 10) and the bacteria have been established within the xylem tissue (Fig. 10). It was noted that some portions of the tumor tissue have been destroyed by the bacteria to form fissures or cavities (Fig. 11) through which the pathogen will ooze from inside the xylem tissue towards outside the tumor (Fig. 12) under favorable conditions to cause new infection.

These findings indicate that knot or tumor formation on olive trees by *P. savastanoi* proceeds in successive steps. After infection, hypertrophy and hyperplasia occurred in host cells which are near from the bacteria and spread progressively outward from

Fig. 1–8. (1) An olive twig affected by the olive knot disease showing tumors. (2) Enlarged tumor from which bacteria are oozing (arrows) outside the knot. (3) Gram stained slide from the oozing bacteria. The pathogen, *Pseudomonas savastanoi*, is a Gram-negative bacterium and rod-shaped. (4) Cross section of part of a healthy olive twig. (5) Beginning of tumor formation. Note cell division, d, of the host tissue at the infection site. (6) Cross section showing the dissolution of host cells which are affected by the presence of the pathogen. Cells around the fissure, fi, are hypertrophied causing pressure upon normal cells beyond them. (7) A more advanced stage of hyperplasia in tumor formation, d. Cells around cavities have been differentiated into disarranged and malformed tracheary elements, t. (8) Cross section through an infected twig in which tumor development has been advanced. Note that the centre of the tumor contains xylem elements, t, surrounded by hyperplastic tissue, d. b = bacteria; c = cortex; d = dividing cells; f = fibers; fi = fissures or cavities; p = pith; pr = periderm; t = tumor tissue; and x = xylem (Safranin-fast green stain).

Fig. 9–12. (9) Bacterial fissures near the xylem tissue. (10) Cross section in which the bacteria have been established within the xylem tissue, b, and most of tumor cells, t, have been transformed into tracheary elements. (11) Portions of the tumor tissue, t, have been destroyed by the bacteria to form fissures, fi. Bacterial cells will move through these fissures from the xylem tissue to outside the tumor as in Fig. 12.

that location. This indicates that tumor tissue may be induced under the influence of the auxin indoleacetic acid (IAA). However, Miller (8), Thimann (11), and Torrey (12) believe that a kinin or a kinetin-like substance under the influence of IAA might be involved in cell ploriferation during tumor development. This remains to be proven.

Production of IAA by *P. savastanoi in vitro* have been reported by several investigators (1,6). Wilson (16) reported that *P. savastanoi* isolates differ in their ability to produce IAA. Isolates that induced the larger tumors produced larger amounts of IAA *in vitro*. Also the longivity of the tumor was correlated with the amount of IAA.

Therefore, the differences in size and stability of the tumors formed in association with *P. savastanoi* on olive might be due in part to *in vivo* production by the pathogen (9,10,15).

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