HISTOPATHOLOGICAL COMPARISON OF GALLS INDUCED BY *ANGUINA TRITICI* WITH GALLS SUBSEQUENTLY COLONISED BY *RATHAYIBACTER TRITICI* IN WHEAT

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Summary. Histopathological differences between seed galls of wheat cv. Mexipak containing only the nematode *Anguina tritici* and those also colonised by the bacterium *Rathayibacter tritici* are described. At early stages of gall development, nematodes were present in both types of gall and the cells forming the galls appeared structurally normal with prominent nuclei. After further development, marked differences between nematode- and bacterially-colonised galls were observed. Bacterially-colonised galls contained a pore through which bacterial cells and gummosis exude. Pores were not observed in normal nematode galls. Normal galls had clear organisation with wide walls, and enlargement and thickening of the cell walls of the outermost cell layer. Whereas bacterial galls had loose cellular structure, with bacteria abundant in gall cavities; in most cases nematodes could no longer be seen. The bacteria were never observed in galls containing eggs or second stage juveniles of the nematode.

Keywords: Nematode-bacterium association, seed gall nematode, *Triticum aestivum*.

Plant-feeding nematodes are damaging to field crops, especially when they act in association with other plant pathogens (Powell, 1971). The association of *Anguina tritici* (Steinbuch) Filipjev with the bacterium *Rathayibacter tritici* (Carlson *et* Vidaver) Zgurskaya *et al*., can cause severe gumming disease of wheat (Bird, 1981). This disease complex occurs in many wheat-growing areas of the world including Iraq (Fattah, 1986) and Syria (unpublished). The presence of *A. tritici* appears to be essential for *R. tritici* to colonise the host (Gupta and Swarup, 1972). When yellow "bacterial galls" are present these are actually bacterially-colonised nematode galls (Riley and Reardon, 1995) and there is no evidence that the bacterium can induce gall formation. Pathak and Swarup (1984) reported the occurrence of the bacterium in 40-55% of the nematode galls and suggested that contaminated nematode juveniles were the main source of the bacterial infection.

This histopathological study was undertaken to compare normal nematode galls with bacterially-colonised galls at different developmental stages to provide further understanding of the association between the nematode and the bacteria inside gall tissue.

MATERIALS AND METHODS

Triticum aestivum L. cv. Mexipak was grown at Tuwaitha, Iraq in 2006 and inoculated at planting with *A. tritici* galls from Bakrajo, Iraq. Various inoculation methods and rates were used to ensure adequate nematode infestation and *R. tritici* colonisation (Fattah, 1988). From spike formation, bacterial colonisation became evident. Galls were dissected from the floral structures at 8, 12 and 16 weeks from planting. Galls were fixed for 24 h in FAA (formalin:glacial acetic acid:alcohol:water - 10:5:50:35), dehydrated through a graded series of ethanol-xylol concentrations, embedded in paraffin wax (melting point 58-60 °C) and sectioned to ~10 µm with a rotary microtome. For histological examination, the sections were either stained with tuluidine blue or saffranin plus fast green (Johansen, 1940) and mounted in Canada balsam.

RESULTS

High incidences (over 90%) of both *A. tritici* infestation, as evidenced by gall development, and *R. tritici* colonisation of both ears and nematode galls were obtained by the inoculation methods used. Early in gall development (8 weeks after nematode inoculation), no apparent differences in gall structure were observed between the nematode and those with bacterial colonisation. Nematodes were observed in both types of gall and the cells forming the gall wall were structurally normal with prominent nuclei (Figs 1A and B). The only difference between the normal and bacterially-colonised galls was the presence of dense masses of bacterial cells surrounding the nematodes in the latter (Fig. 1B). Upon further development (12 weeks after nematode inoculation), marked differences were evident between the gall types. Bacterially-colonised galls were partially covered with a bright yellow mass of *R. tritici* cells (Figs 1B, C and Fig. 2). Histological examination also revealed the occurrence of a pore at the top of most bacterially-colonised galls (Fig. 1C). Normal nematode galls showed clear histological organisation with considerable width of gall wall and thickening of the cell walls of the peripheral outermost layer of cells (Fig. 1D). The outer-

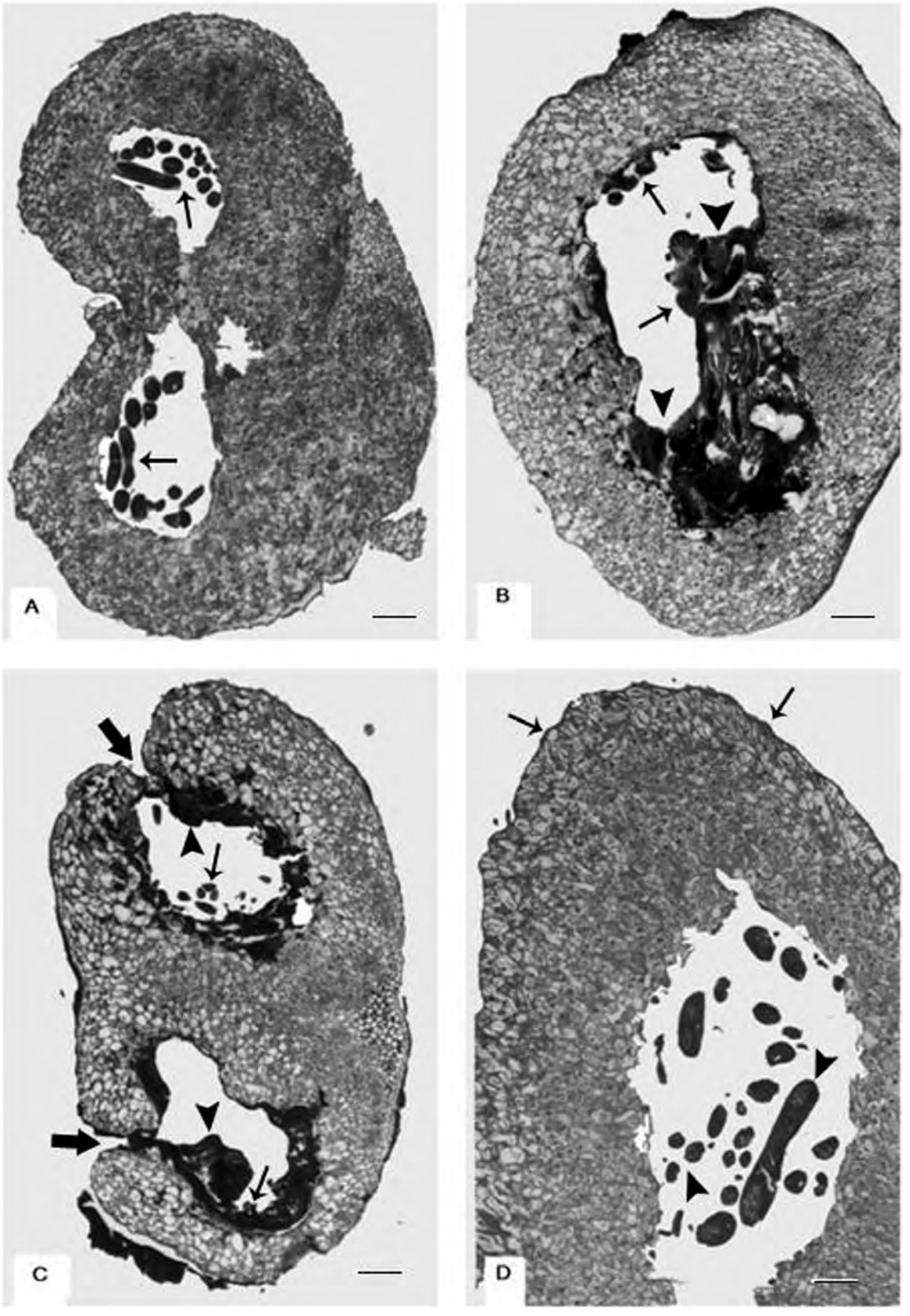

Fig. 1. Section through nematode seed galls induced by *Anguina tritici* in wheat, *Triticum aestivum* cv. Mexipak. Bars = 100 µm. A: eight weeks after nematode inoculation. Note the presence of nematodes inside the gall lumens (arrows). B: 8 weeks after nematode inoculation showing *Rathayibacter tritici* cell mass (arrow heads) around the nematodes (arrows) inside the gall lumen. C: twelve weeks after nematode inoculation showing *A. tritici* (small arrows) and *R. tritici* (arrow heads) within the nematode seed gall. Note the pores at the top of each gall lumen (large arrows). D: 12 weeks after nematode inoculation showing the enlargement of cells and the thickening of the cell walls of peripheral outermost layer of cells of the nematode seed gall (arrows). Note the sectioned nematodes within gall lumen (arrow heads).

most layers (3-4 cells) in the gall wall were composed of large cells with apparently decomposing protoplasm, whereas the cells close to the gall lumen were smaller and contained dense cytoplasm (Fig. 1D). At advanced stages of gall development (16 weeks after inoculation), the bacterially-colonised galls appeared to have loose cellular structure with the exception of the packed and apparently dividing cells at the gall-spike junction (Fig. 2). At this stage, the bacteria were abundant in the gall lumen (Figs 2 and 3B) and nematodes were mostly no longer evident within these galls; if present, they appeared in a degenerated state. The cells of nematode-colonised galls at this stage appeared structurally normal (Fig. 3A) compared to those of normal nematode galls. The bacteria were also observed within gall tissue and in the intercellular spaces surrounded by degenerating cells (Fig. 3B). Furthermore, the bacteria were also observed as connected groups forming a network of cells (Fig. 3B).The bacteria were not observed in sectioned galls that contained eggs or second stage juveniles of the nematode (Figs 3C, D). Although bacterially-colonised galls are induced by the nematode, they become totally occupied by the bacterium, which displaces the nematode and prevents it from developing.

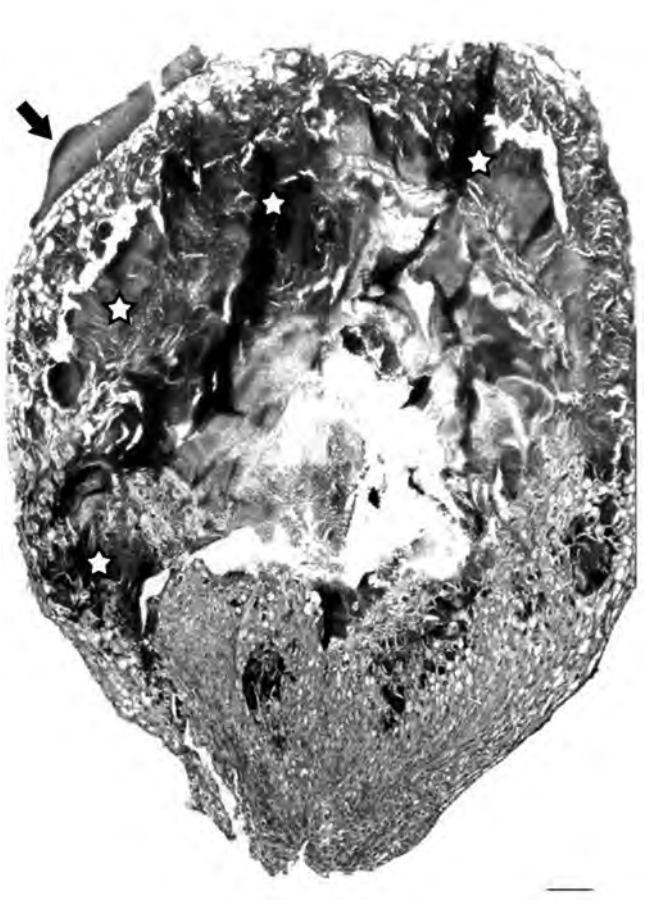

Fig. 2. Section through nematode seed gall induced by *A. tritici* in wheat, *T. aestivum* cv. Mexipak, at advanced gall developmental stage (16 weeks after nematode inoculation). Note the enlargement and the thickening of the peripheral cells and the mass of *R. tritici* cells and gummosis within the gall tissue and lumen (stars) and partially covering the gall (arrow). Bar = 800 μm.

DISCUSSION

The presence of a pore at the top of bacterially-colonised galls provides a path through which bacterial cells exude outside the gall lumen (Fig. 1C). This explains the partial covering of the galls with gummosis as the infection develops. This fact also explains the shriveled and disorganized appearance of the bacterially-colonised galls (Suryanarayana and Mukhopadhya, 1971; Fattah, 1986). The occurrence of such openings in the bacterially-colonised galls was not reported in the *Anguina funesta* Price, Fisher *et* Kerr-*Rathayibacter toxicus* (Riley *et* Ophel) Sasaki *et al.* association in *Lolium rigidum* Gaud (Bird *et al.*, 1980). The clear histological organisation and the considerable width and thickening of the cell walls of the outermost layer of the gall wall (Fig. 1D) was also reported for galls induced by *A. funesta* (Stynes and Bird, 1982). The outermost layers in the normal nematode galls (Fig. 1D) are probably essential for the nematode gall integrity and the protection of the anhydrobiotic second stage juveniles over the hot and dry summer. The inner cells (Fig. 1D) closest to the feeding nematodes (adults) may provide the nutrients needed for nematode development and subsequent reproduction. It was demonstrated that cells adjacent to the gall cavity were densely packed with organelles, suggesting a high state of metabolic activity (Midha and Swarup, 1974).

At advanced stages of gall development (16 weeks after nematode inoculation) the proliferation of bacteria (Figs 2, 3B) created an abnormal environment, preventing nematode development and reproduction. It has been suggested that the bacteria that are carried in or on apparently normal galls are sufficient to ensure *R. tritici* survival (Riley and Readon, 1995), and play a major role in the ecology of *R. tritici-A. tritici* association. This was also evident from the bacterial infection that arose from intact seed gall inoculation in this study and previous work (Gupta and Swarup, 1974; Fattah, 1988; Riley and Readon, 1995). The observation of groups of bacteria as a network of cells (Fig. 3B) is most likely due to the filamentous materials that cover *Rathayibacter* capsules (Bird, 1981). The filamentous nature of these bacteria is considered to be important in connecting bacterial cells together and allowing them to adhere to compatible *Anguina* spp. The bacterial adhesion is not about host specificity of the nematode but rather the partial-specificity of the bacteria-nematode associations (Riley, 1992).

The apparent absence of bacteria in galls containing eggs and/or second stage juveniles of the nematode (Figs 3C, D) and the fact that galls may be occupied by either organism is due to undetermined factors and is probably important for the co-existence of *R. tritici* with this nematode.

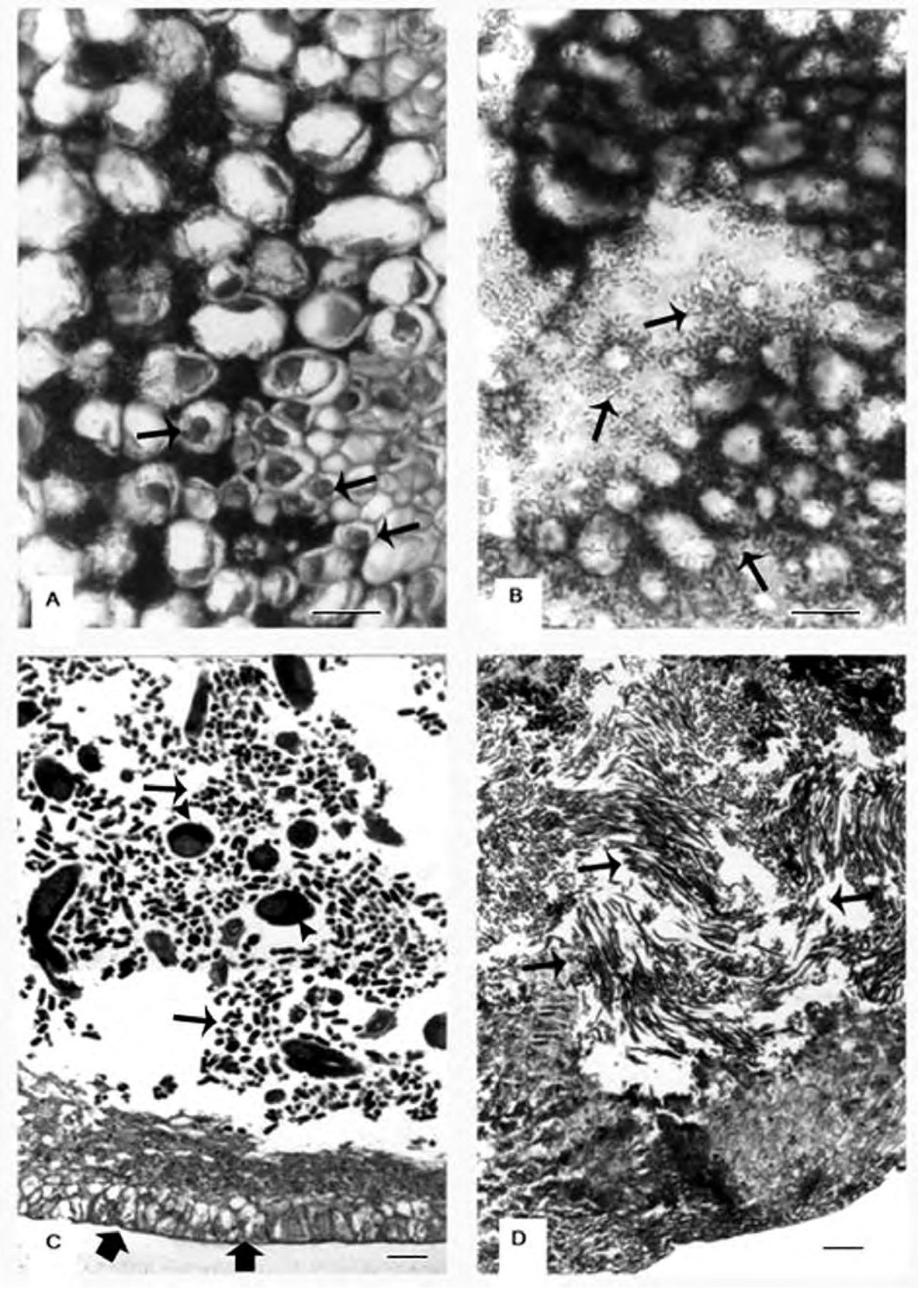

Fig. 3. Sections through seed galls induced by *A. tritici* in wheat, *T. aestivum* cv. Mexipak, 16 weeks after nematode inoculation. Bars in A and B = 300 µm. A: normal looking gall tissue showing cells with prominent nuclei (arrows). B: large number of *R. tritici* cells forming networks present in degenerating gall tissue and in the intercellular spaces (arrows). C: section through large number of eggs (arrows) and adult nematode (arrow heads). Note the well developed gall wall and the thickened wall of the cells at the outermost layer of cells (large arrows). Note the absence of bacteria. Bar = 400 µm. D: large number of nematode juveniles in fully mature nematode seed gall (arrows). Note the absence of bacteria. Bar = 100 µm.

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