Electrolyte Leakage and Membrane Damage in Relation to Bacterial Population, pH, and Ammonia Production in Tobacco Leaf Tissue Inoculated with Pseudomonas pisi

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ABSTRACT

When 10^8 cells/ml *Pseudomonas pisi* were injected into tobacco foliage which was subsequently kept in either a normal room or humid chamber atmosphere, typical visual symptoms of the hypersensitive reaction (HR) (flaccidity and necrosis) developed only in the former environment. The bacterial population decreased 12 hr after inoculation in tissue held in room atmosphere, and increased in the humid chamber during the 48 hr of the experiment. Membrane damage and electrolyte leakage, consequences of HR, occurred in both environments approximately 6 hr after inoculation. This is 18 hr before the pH reaches 8.0, and 42 hr before NH_3 is evolved; hence, neither is causally related to either membrane damage or electrolyte leakage. The evolution of NH_3 only at 48 hr is a reflection of continued metabolic activity of the bacteria in the humid chamber environment.

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The hypersensitive reaction induced by *Pseudomonas pisi* and other incompatible plant-pathogenic bacteria in tobacco is characterized by a rapid desiccation of inoculated tissues (3, 4, 5). The injection (3) of 10^8 cells/ml of *P. pisi*, twice washed, into tobacco foliage causes flaccidity in 6 hr and almost complete desiccation 18 hr after inoculation. At 6 hr after inoculation, electrolyte leakage is intense (1), and electron micrographs (2) reveal extensive membrane damage to subcellular organelles, tonoplast, and plasmalemma.

In order to monitor electrolyte leakage, pH, and membrane changes in the tissue more precisely and for a longer period of time, I attempted to delay the desiccation process; this was done by inducing the hypersensitive reaction (HR) in tobacco with 10^8 cells/ml of *P. pisi* and permitting the phenomenon to proceed in a petri dish humid chamber (5). Under these experimental conditions, desiccation could be delayed for more than 48 hr. At ca. 48 hr after inoculation, large quantities of NH_3 (which can be trapped in H_2SO_4) are evolved from the inoculated leaf tissue. A rise in tissue pH to 8.0-8.5 also occurs at or just prior to that time (5). As a consequence, we reported that the rise in pH and NH_3 evolution were causally related to HR (5).

The experiments described in this communication sought to determine whether (i) delayed desiccation of inoculated tobacco leaves in the petri dish also reflected a delay or suppression of electrolyte leakage; (ii) ultrastructural damage was delayed in tissue maintained in the petri dish; and (iii) the population patterns of bacteria in leaf tissue kept inside the petri dish or exposed to the atmosphere differed appreciably.

The data concerning electrolyte leakage, pH, and bacterial populations are presented in Fig. 1 and are representative of values obtained in four separate

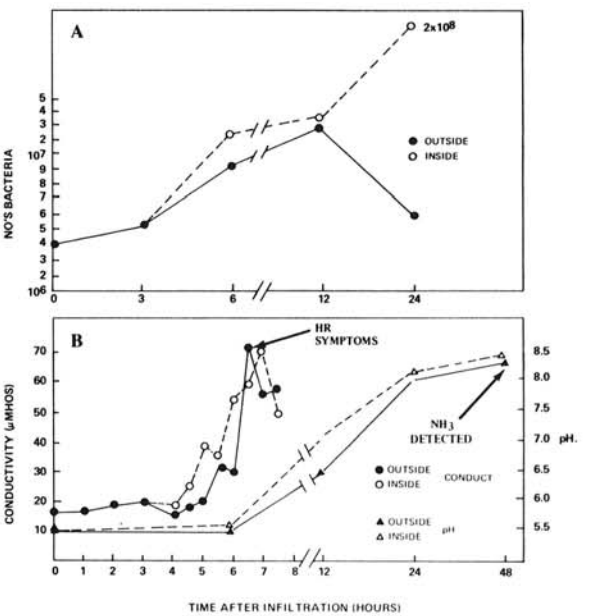


Fig. 1. A) Influence of 10^8 cells/ml *Pseudomonas pisi* injected into tobacco foliage kept subsequently in normal room (outside) and humid chamber (inside) atmospheres on bacterial populations; **B)** conductivity (electrolyte leakage) and pH. HR=Hypersensitive reaction.

experiments. It is apparent that electrolyte leakage occurs in tissue maintained "inside" as well as "outside" the petri dish at approximately the same time (1). Furthermore, electrolyte leakage peaks in both instances approximately 42 hr before NH_3 is detected in the petri dish environment. We reported earlier (5) that a rise in pH to 8.0 or higher was causally related to the release of NH_3 as a gas from

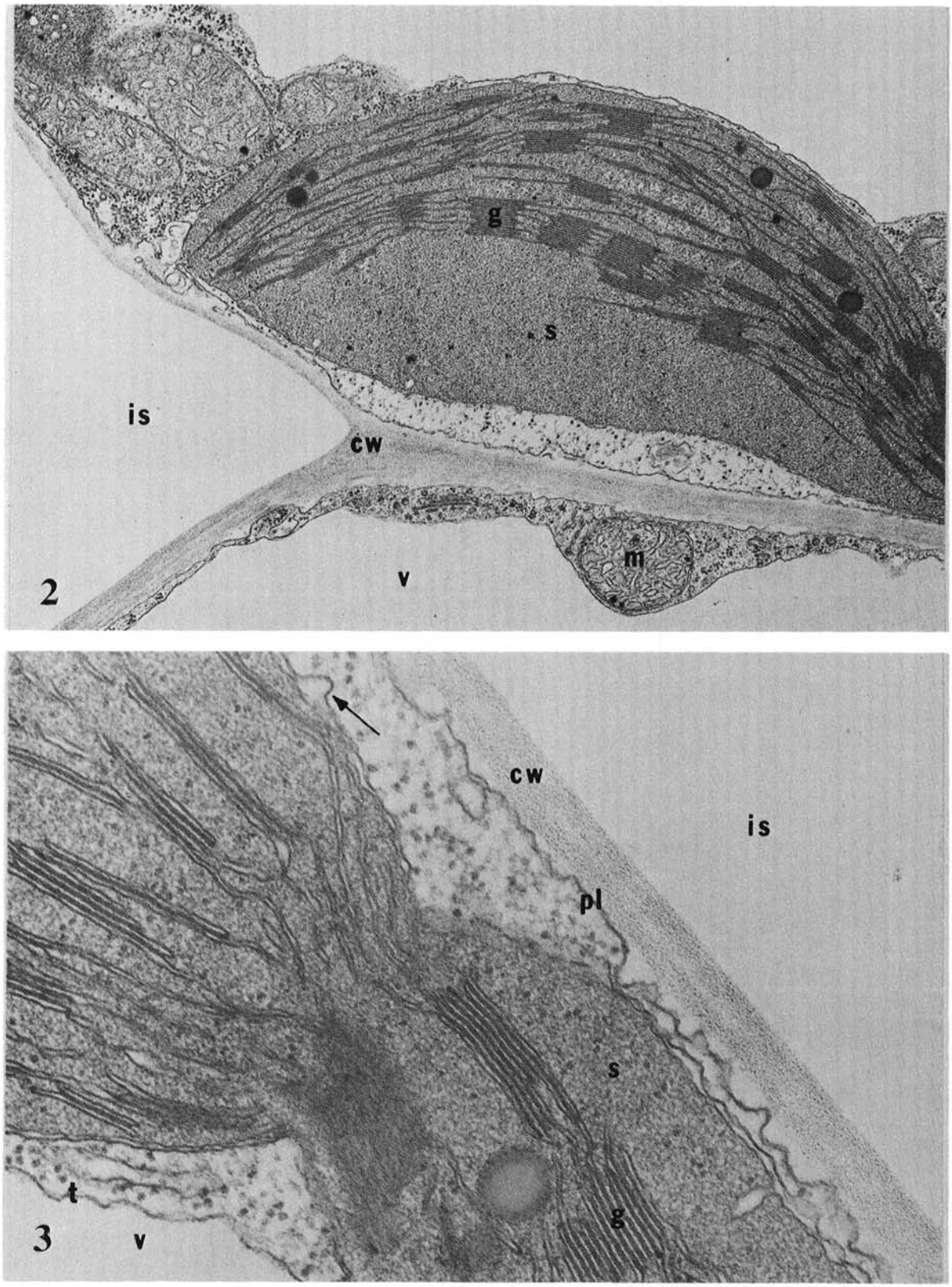


Fig. 2-3. Tobacco leaf tissue fixed 6 hr after treatment. **2)** Appearance of subcellular organelles is normal in water-injected control tissue (× 18,000). **3)** Outer membrane of the chloroplast (arrow), plasmalemma, and tonoplast are all clearly continuous and in close proximity to the cell wall in control tissue (× 58,500); cw = cell wall; g = grana; is = intercellular space; m = mitochondrion; pl = plasmalemma; s = stroma; t = tonoplast; v = vacuole.

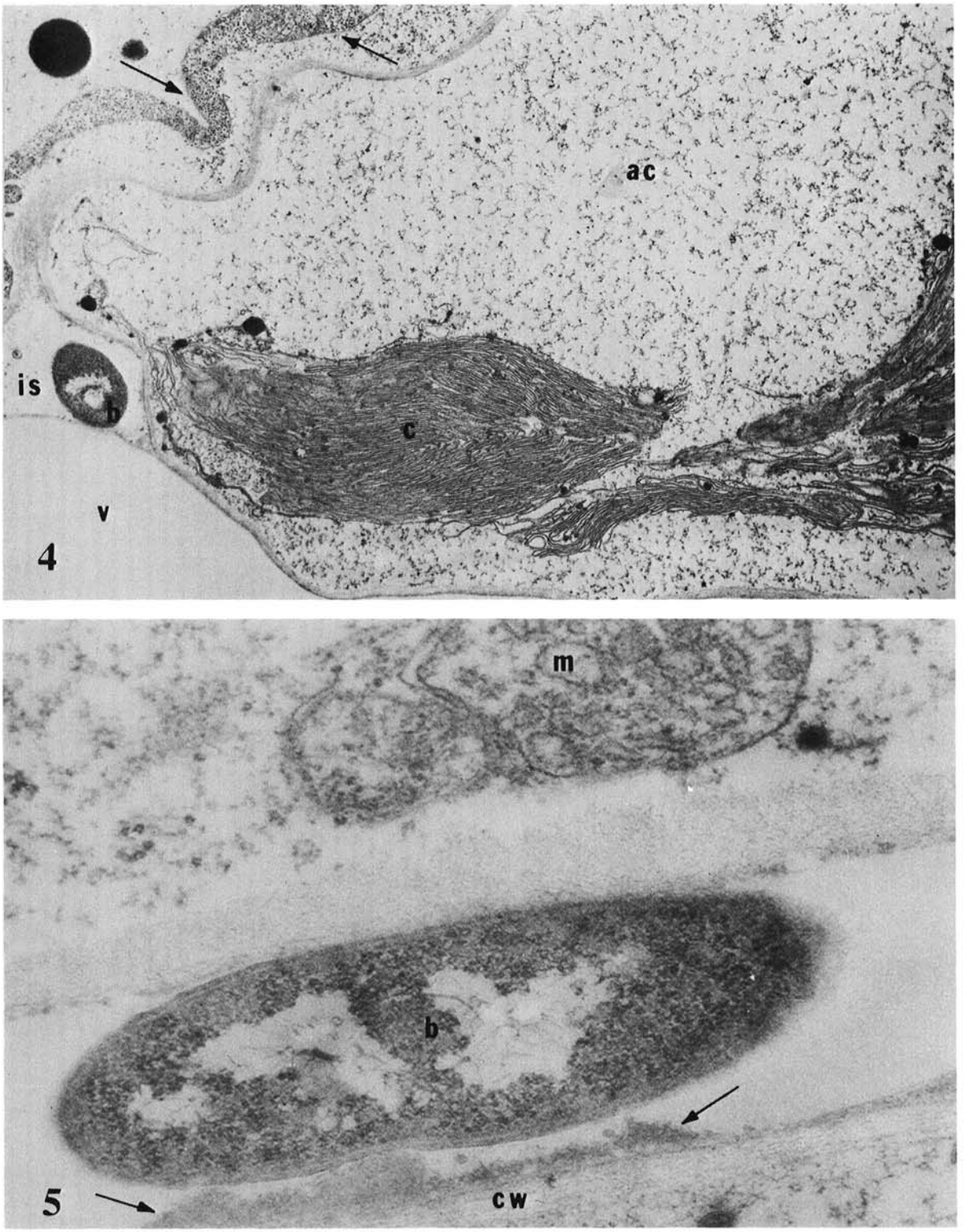
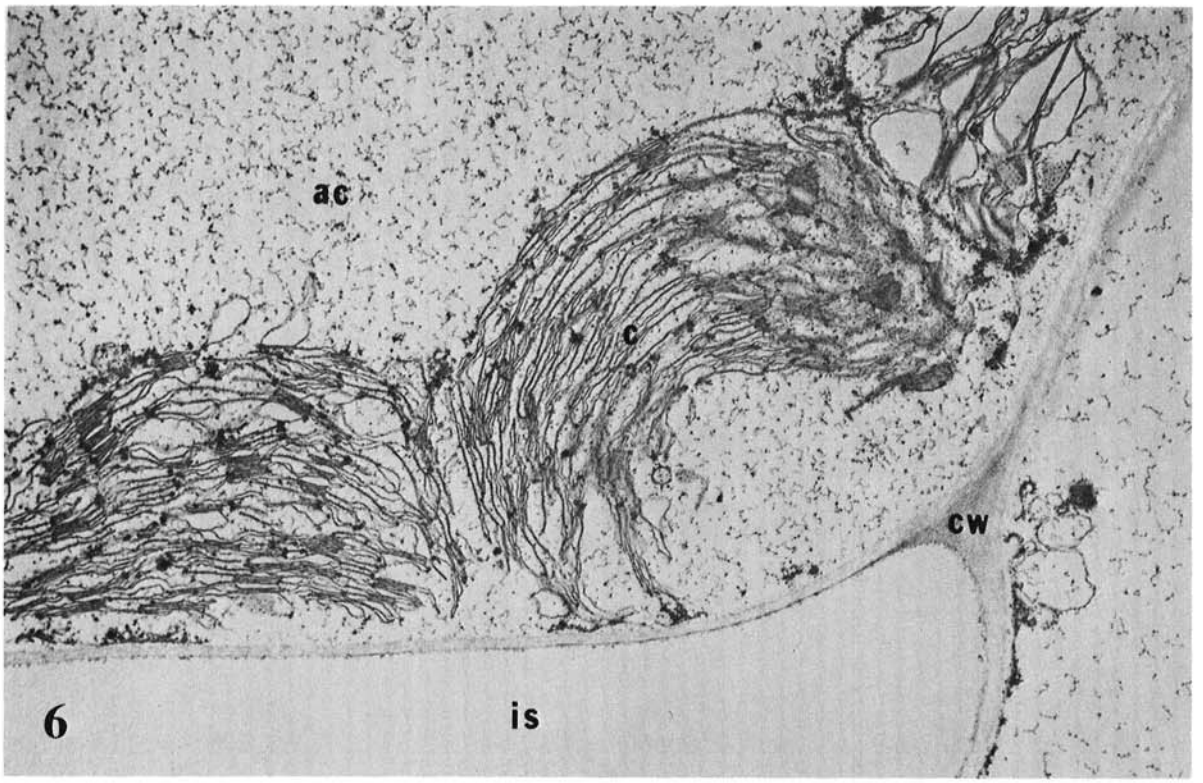


Fig. 4-5. Tobacco leaf tissue fixed 6 hr after treatment. **4)** Bacteria-injected tissue kept "outside"; note loss of stroma and bounding membrane of chloroplast; diffuse cytoplasmic aggregates and arrows indicate plasmalemma and tonoplast bounding vestigial mitochondrion (arrow); all of which have pulled away from the cell wall (× 13,200). **5)** Tissue ("outside") injected with bacteria showing bacterial cell with adjacent electron-dense material (arrows) of unknown origin between it and "fibrillar" cell wall; note also vestigial mitochondrion and absence of plasmalemma (× 70,200); ac = aggregated cytoplasm; b = bacteria; c = chloroplast; cw = cell wall; is = intercellular space; m = mitochondrion; v = vacuole.



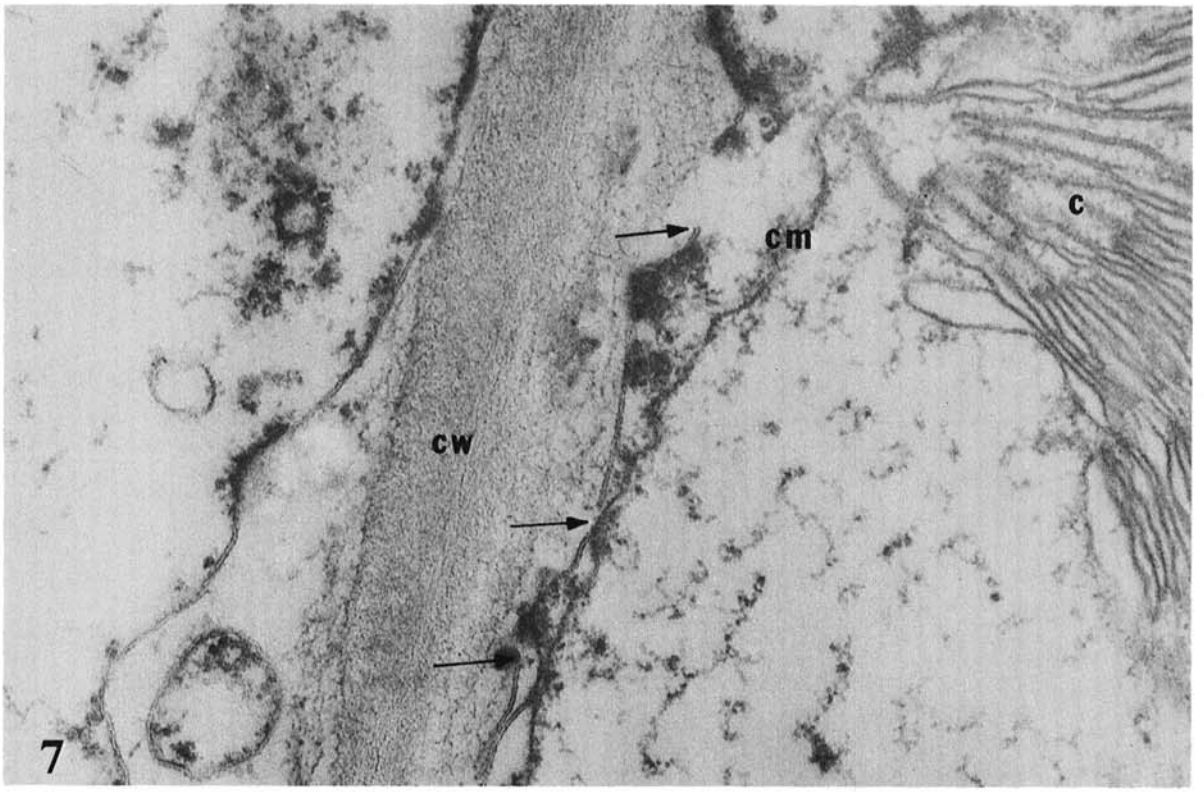


Fig. 6-7. Tobacco leaf tissue fixed 6 hr after treatment. **6)** Bacteria-injected tissue kept "inside", the disorientation of chloroplasts is even more intense than in Fig. 4 (× 13,200). **7)** Tissue ("inside") injected with bacteria showing "doubletrack" plasmalemma broken at arrows and pulled away from the "fibrillar" wall in cell at left. The outer chloroplast membrane is diffuse and distant from the main body of grana (compare with Fig. 3) (× 59,400). ac = aggregated cytoplasm; c = chloroplast; cm = chloroplast outer membrane; cw = cell wall; is = intercellular space.

inoculated tissue. The current data indicate that this pH rise does in fact occur, and it precedes NH_3 release. However, this pH is reached at least 18 hr after electrolyte leakage has peaked and HR symptoms are visible in leaf tissue maintained in the "outside" environment.

The data pertaining to bacterial populations (monitored by standard plate count procedures performed in triplicate) inside and outside reveal a slightly potentiated rate of growth of *P. pisi* inside the petri dish during the first 12 hr, which may account for the earlier rise in electrolyte leakage detected in this environment (Fig. 1). However, the real divergence in population trends between the two environments occurs between 12 and 24 hr. *Pseudomonas pisi* kept inside continues to multiply rapidly, whereas the bacteria are dying in tissue which is kept outside and which is undergoing rapid desiccation characteristic of HR.

Electron micrographs of tobacco leaf tissue obtained 6 hr after infiltration with 10^8 cells of *P. pisi* disclose membrane damage that is similar and as extensive in tissue kept in either the "inside" or "outside" environment (Fig. 2-7). It is clear, therefore, that membrane damage occurred well before the time when NH_3 was detected or the rise to pH 8.0 occurred. The plant material was prepared for electron microscopy precisely as previously described (2).

The vast quantities of NH_3 evolved from leaf tissue (980 μg/g fresh wt) detected (5) 48 hr after inoculation only in the closed system is interpreted as a reflection of metabolic activity of the bacteria which are continuing to grow under these conditions. Analyses at 3-hr intervals of inoculated leaf tissue kept either inside or outside the petri dish failed to reveal increases in NH_3 over controls during the 48-hr duration of the experiment.

From the data and observations reported herein, I have concluded that delaying HR symptoms in the petri dish atmosphere delays neither electrolyte leakage nor membrane damage. Furthermore, since these two phenomena occur long before NH_3 is detected or the pH rises above 8.0, I have concluded that neither NH_3 nor the rise in pH is causally related to HR. A portion of data presented herein contradict conclusions drawn by us earlier (5).

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