Effect of Bacterial Infiltration on Photosynthesis of Bean Leaves

Andrew C. Magyarosy and Bob B. Buchanan

Former Research Assistant and Professor, Departments of Plant Pathology and Cell Physiology, respectively, University of California, Berkeley 94720. Present address of senior author: United States Department of Agriculture, Salinas, California 93901.

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ABSTRACT

Bean leaves were infiltrated with a suspension (10^9 cells/ml) of *Pseudomonas phaseolicola,* a virulent pathogenic bacterium (compatible combination), *Pseudomonas fluorescens,* a nonpathogenic or saprophytic bacterium, or with distilled water (control treatment). A loss of turgor was observed 8-10 hours after infiltration with the pathogen, analogous to the sequence of events reported for incompatible combinations (hypersensitivity). Changes in capacity for photosynthetic $^{14}CO_2$ assimilation and in chloroplast ultrastructure were monitored as a function of time after infiltration. The results indicate that (i) the pathogen causes a progressive decrease in the rate of photosynthesis that is followed by destruction of chloroplast membranes; (ii) the saprophyte causes a sharp transitory reduction in the rate of photosynthesis that is not associated with a structural change in chloroplasts; (iii) neither the pathogen nor the saprophyte alters photosynthetic products in leaves.

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It has been established that certain pathogenic bacteria induce in resistant plants (incompatible combination) a hypersensitive reaction that leads within hours to loss of turgor and to leaf collapse and necrosis (1, 6, 10, 11, 12). A similar infiltration by water or by nonpathogenic (saprophytic) bacteria has little effect on either the appearance or the growth of the plant.

Early studies on physiological changes accompanying the hypersensitive reaction revealed a rapid loss of electrolytes from hypersensitive tissues that was ascribed to disruption of cell membranes (3, 4, 6). More recent investigations based on ultrastructural changes accompanying leaf infiltration indicate severe damage to chloroplasts after several hours (7). These findings have prompted the conclusion that an early effect of the hypersensitive reaction is the destruction of membranes of chloroplasts and other cell organelles (8, 9). However, there is little evidence on the physiological or ultrastructural effects of pathogenic bacteria infiltrated in large numbers into susceptible plants (compatible combination).

To obtain evidence on this point, we investigated the effect of infiltration of a virulent bacterium into bean leaves (15). Leaves were infiltrated with either the pathogenic bacterium *Pseudomonas phaseolicola* (Burk) Dawson., isolate HB41, or the saprophytic bacterium *Pseudomonas fluorescens* (Trev) Migula., isolate 18, and monitored for changes in capacity for photosynthetic $^{14}CO_2$ assimilation and in chloroplast ultrastructure. A preliminary account of these findings has been published (13).

MATERIALS AND METHODS.—Cultures of the pathogen *Pseudomonas phaseolicola,* isolate HB41, which is virulent on Pinto bean (15), and the saprophyte *Pseudomonas fluorescens* isolate 18 were maintained as a lyophilized powder and grown in King's medium B slants at 29 C. Prior to infiltration, cells of 1-day-old cultures were suspended in sterile distilled water and centrifuged at 12,100 *g* for 1 minute. The supernatant fluid was discarded and the pellet was washed twice as above and resuspended in distilled water. The suspension used for filtration contained 10^9 cells/ml, as determined in a Klett colorimeter by relating absorption of the suspension to a standard curve obtained with known numbers of cells. Heat-killed saprophytic bacteria were obtained by autoclaving the cell suspension for 20 minutes (120 C, 1.3 atm).

Plants *Phaseolus vulgaris* L. (Pinto bean) were grown in UC mix under normal greenhouse conditions (2). One of the two primary leaves of a 14-day-old plant was infiltrated under vacuum with a suspension of either the pathogen, the viable saprophyte, or the autoclaved saprophyte. The opposite leaf of each plant served as the control and was infiltrated with sterile distilled water. After infiltration, plants were placed in a growth chamber at 20 C under an illumination of 21,520 lx until samples were taken for measurements of photosynthetic CO_2 assimilation or for study of chloroplast ultrastructure.

Photosynthetic $^{14}CO_2$ assimilation was carried out 2, 6, 10, and 16 hours after infiltration with duplicate leaf disks, one being used for counting and the other for product analysis (14). Disks were floated stomata-side-up in Warburg vessels containing 1.5 ml of H_2O in the main compartment and 0.1 ml $NaH^{14}CO_3$ (10 μmoles, 5×10 cpm/μmole) in a sidearm fitted with a serum cap. After 5

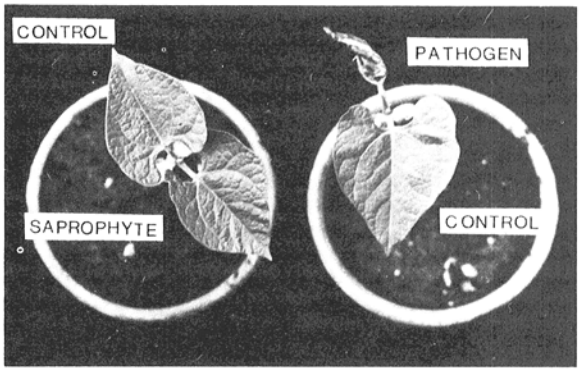

Fig. 1. Effect of bacterial infiltration on bean leaves. Leaves were infiltrated as indicated with the pathogen, the saprophyte, or distilled water (control treatment); photograph was taken 16 hours after infiltration.

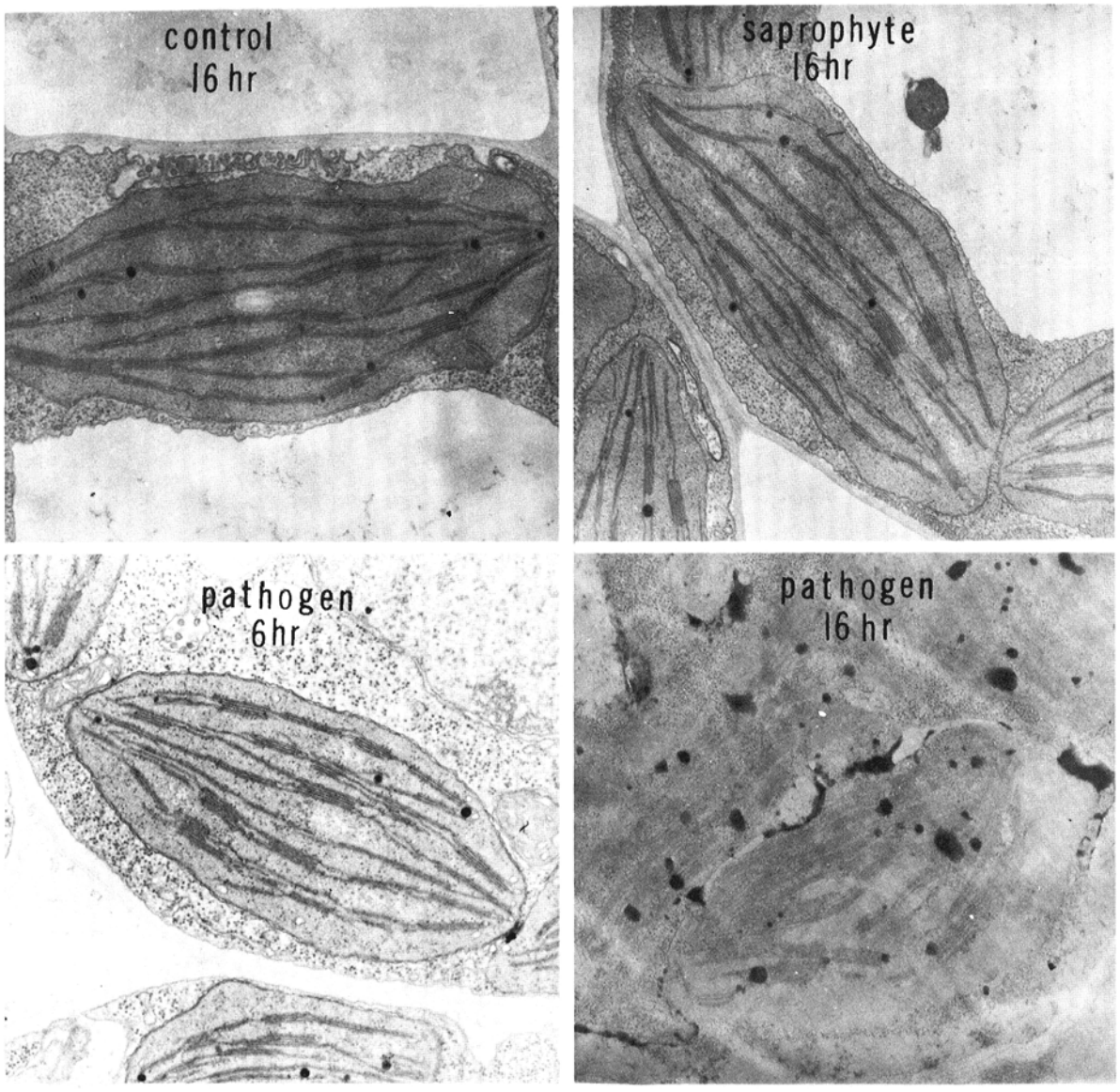

Fig. 2. Effect of bacterial infiltration on chloroplast ultrastructure. Leaves were infiltrated as indicated with the pathogen, the saprophyte, or distilled water (control treatment) and embedded at the indicated times after infiltration for electron microscopy. ×28,808.

minutes of preillumination, 0.2 ml of 9N H_2SO_4 was injected into the sidearm to liberate gaseous $^{14}CO_2$ to start the reaction. Photosynthesis was stopped by immersing the leaf disks in boiling 80% ethanol for 2 minutes. The rate of CO_2 assimilated (μmoles/mg/chlorophyll/hour) was determined 6 minutes after liberating $^{14}CO_2$ and compared to the water-infiltered control.

Electron micrographs were prepared from infiltrated leaves that had been fixed in a 2% glutaraldehyde mixture and dehydrated, sectioned, and stained with uranyl acetate as described by Esau (5). Sections of leaves harvested 2, 6, and 16 hours after infiltration were examined with a Siemens Elmiskop 101 electron microscope.

RESULTS AND DISCUSSION.—Under our experimental conditions, loss of turgor was first apparent 8-10 hours after infiltration with the pathogen. Such loss became progressively more acute and ultimately led to total necrosis. It should be pointed out that this sequence of events is similar to that reported for incompatible combinations; i.e., hypersensitivity. The relation of the reaction observed after bacterial infiltration in compatible combinations to that for incompatible combinations therefore becomes an important question.

Fig. 1 shows bean plants 16 hours after infiltration with the pathogen, the saprophyte, or distilled water (control treatment). Whereas no visible change was produced by the saprophyte, infiltration with the pathogen caused a

loss of leaf turgor and profuse necrosis. Analogous effects were observed in chloroplast ultrastructure: 16 hours after infiltration the saprophyte effected no change, whereas the pathogen caused extensive disintegration of chloroplast membranes (Fig. 2). It is noteworthy that no structural damage to chloroplasts was detected up to 6 hours after infiltration with the pathogen. This finding is to be compared with the observation of Goodman and Plurad (7) that, in an incompatible combination, a profound derangement in membranes of chloroplast microbodies and other organelles occurred prior to 7 hours after infiltration.

Despite the absence of structural damage to chloroplast membranes before 6 hours after infiltration in our compatible combination, the pathogen induced after 2 hours a reduction in the rate of photosynthetic CO_2 assimilation that progressively became more pronounced but did not become complete within 16 hours (Fig. 3). Even after the development of profuse necrosis the rate of CO_2 assimilation in pathogen-infiltrated leaf disks was at least 15% that of the water-infiltrated control.

A different response was observed with leaf disks of the saprophyte-infiltrated plant. The rate of CO_2 assimilation dropped sharply 2 hours after infiltration, and gradually returned to the control level after 14 hours (Fig. 3). Viable saprophytic cells were not required for this effect; as shown in Table 1, the autoclaved saprophyte also brought about a reduction in the rate of CO_2 assimilation. It would therefore appear that an inhibitor produced in situ by the viable infiltrated saprophyte is not responsible for the observed drop in the rate of photosynthesis. It is noteworthy that, despite the reduction in the rate of photosynthetic CO_2 assimilation in each case, neither the pathogen, the viable saprophyte, nor the autoclaved saprophyte consistently changed photosynthetic products formed by leaf disks (Table 2). It would, therefore, appear that these agents do not selectively suppress individual reactions of CO_2 assimilation.

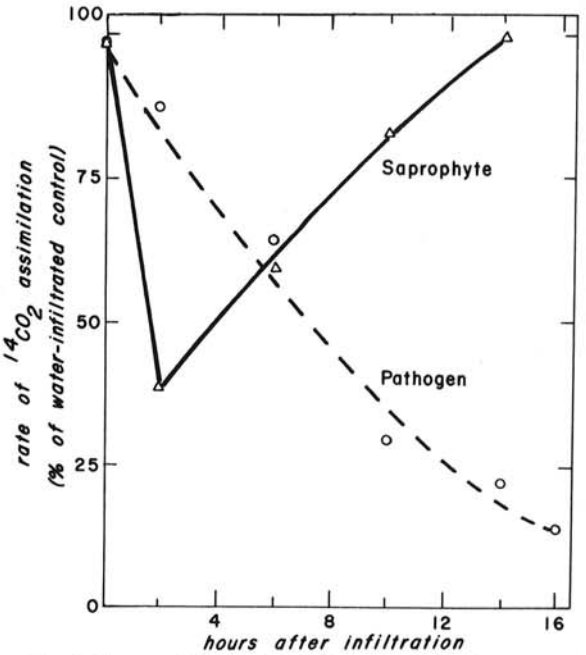

Fig. 3. Effect of bacterial infiltration on the rate of photosynthetic $^{14}CO_2$ assimilation by leaf disks. Each point represents the mean rate (eight replications) of photosynthesis (μmoles/mg chlorophyll/hour) 6 minutes after liberating $^{14}CO_2$. The rates of the water-infiltrated control disk were 72-86 μmoles of $^{14}CO_2$ fixed/mg chlorophyll per hour for the times shown.

TABLE 1. Effect of infiltration with viable and autoclaved saprophytic bacteria on the rate of photosynthetic $^{14}CO_2$ assimilation by bean leaf disks[a]

Hours after infiltration	CO_2 fixed (μmoles/mg chlorophyll/hour)			Ratio of CO_2 fixed (autoclaved:viable saprophyte)
	Control	Viable saprophyte	Autoclaved saprophyte	
2	88	50	30	0.6
6	76	58	40	0.7
10	74	59	54	0.9

[a]Experimental conditions were as described for Fig. 3. Values represent the mean of six replications.

TABLE 2. Effect of bacterial infiltration on photosynthetic products formed by bean leaf disks[a]

	Total $^{14}CO_2$ fixed (%)			
	Water-infiltrated control	Pathogen	Viable saprophyte	Autoclaved saprophyte
Organic acids	4.0	4.8	5.5	3.5
Amino acids	18.0	17.8	17.7	18.5
Sugars + sugar phosphate	49.0	56.9	56.4	48.7
Starch and other neutral compounds	13.5	11.2	8.3	12.1
Other compounds	15.5	9.3	12.1	17.2

[a]Disks were assayed 6 hours after infiltration with the indicated agent. Products were analyzed for each disk after 6 minutes of photosynthesis under the conditions given in Materials and Methods section. The values represent the mean of six replications.

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