Growth Kinetics and Histopathology of *Xanthomonas campestsris* pv. *glycines* in Leaves of Resistant and Susceptible Soybeans

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ABSTRACT

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In soybeans, a very high level of resistance to the bacterial pustule disease is conferred by a recessive gene designated *rxp*. Studies were undertaken to characterize this resistance mechanism. The first trifoliolate leaves of resistant and susceptible soybean plants were inoculated with *Xanthomonas campestris* pv. *glycines* by using an airbrush. Inoculum concentrations were varied to produce initial bacterial populations ranging from 5 to 1,600 colony-forming units (cfu)/cm^2, and pathogen populations were assessed over a 9-day period. At all inoculum concentrations, pathogen populations increased at similar rates in both resistant and susceptible leaves. The mean generation time was 10.49 hr. For both susceptible and resistant cultivars, the number of pustules formed 10 days after inoculation was proportional to the inoculum concentration. The number of pathogen cells required to initiate a single pustule was at least six times greater in the resistant cultivars than in the susceptible cultivars. Pustules, which resulted from hypertrophy of mesophyll cells, were smaller on resistant cultivars. Anatomical responses to infection were similar in resistant and susceptible cultivars, although the responses were of somewhat reduced intensity in the resistant plants. These results indicate that the *rxp* gene confers resistance, at least in part, by increasing the number of bacterial cells necessary for infection rather than by restricting pathogen growth within host tissues.

Additional key words: infectivity titration, *Glycine max*.

The bacterial pustule disease of soybeans (*Glycine max* (L.) Merr.), caused by *Xanthomonas campestris* pv. *glycines* (Nakano) Dye, is characterized by small yellow-to-brown lesions with a raised pustule in the center. The lesions are restricted to the leaves and may merge to form large necrotic areas. A very high level of resistance to bacterial pustule is conditioned by a single recessive gene, designated *rxp* (2), originally found in the cultivar CNS (13,19). This resistance has endured for more than 30 years in spite of the fact that it is the only widely used source of resistance and it has been incorporated into virtually all soybean cultivars grown in the South and many cultivars grown in the Midwest (2).

Resistant plants rarely become infected under natural conditions in the field. When resistant cultivars are artificially inoculated, however, substantial pathogen growth occurs, and plants can become severely diseased (4,14). It seemed unusual to us that such a highly effective and durable type of resistance was so easily overcome after artificial inoculation. Studies were initiated to characterize the mechanism of bacterial pustule resistance mediated by the *rxp* gene. Fett (14) has recently demonstrated that phytoalexins are not involved in resistance in the cultivar Clark 63. This paper reports the results of comparative studies of pathogen growth kinetics and histopathology in resistant and susceptible soybeans. A preliminary report of this work has been published (16).

MATERIALS AND METHODS

Plant material, pathogen strains, and inoculation techniques. Four susceptible cultivars (Clark, Oakland, BSR 301, and Harosoy) and five resistant cultivars containing the *rxp* gene (Clark 63, BSR 302, Williams, L61-4180, and L61-4094) were used in these studies. Clark and Clark 63 are near-isogenic cultivars. Clark 63 has resistance to *X. c.* pv. *glycines* and to race 1 of *Phytophthora megasperma* var. *sojae*. Two other pairs of near-isogenic lines, Clark and L61-4180 and Harosoy and L61-4094, were obtained from R. L. Bernard, United States Regional Soybean Laboratory, Urbana, IL.

The isolate of *X. c.* pv. *glycines* used (Xcg-8) was obtained from an infected soybean plant (cv. BSR 301) near Ames, IA, in 1980 and was characterized as a pathovar of *X. campestris* according to the criteria of Dye (10). To provide uniform inoculum for all experiments, actively growing cells derived from a single colony of typical morphology were suspended in sterile 5% skim milk, subdivided, and frozen in liquid nitrogen. Inoculum was produced by rapidly thawing frozen cells and spreading them on a yeast extract and calcium carbonate medium (YCA) containing 20 g of agar, 10 g of yeast extract, and 2.5 g of calcium carbonate per liter of distilled water. After growth for 24–28 hr at 20 C, cells were suspended in sterile phosphate buffer (pH 7), adjusted to a density of 10^8 colony-forming units (cfu) per milliliter with a spectrophotometer, and diluted to the desired concentration with sterile buffer.

All inoculations were made on recently expanded first trifoliolate leaves of 10- to 14-day-old plants. Plants were placed in a moist chamber for 1–2 hr before inoculation. An airbrush was then used to spray inoculum suspensions against the undersides of the leaves until watersoaking appeared. Initial pathogen populations were determined by the leaf-grinding and dilution-plating methods described below. After inoculation, plants used in infectivity titrations were held in a greenhouse (26 C, natural light). All other plants were placed in a growth chamber (26 C, 16-hr day length, 17,000 lx).

Growth assessment. Bacterial populations were assessed at 0, 1, 3, 5, 7, and 9 days after inoculation by using standard dilution-plating techniques. Samples consisted of 10 0.24-cm disks (two disks from each of five plants) taken from the inoculated area of the leaf. Disks were briefly dipped in 70% ethanol, washed in sterile

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distilled water, and ground in sterile phosphate buffer containing 0.1% Triton X-100 (Fisher Scientific, Fair Lawn, NJ). Samples were then serially diluted and plated on YCA. Pathogen growth was examined in Clark and Clark 63 at initial inoculum concentrations of 5, 70, and 1,600 cfu per square centimeter of leaf tissue and in BSR 301 and Williams at an initial concentration of 5 cfu/cm^2. There were five inoculated plants in each cultivar/inoculum concentration treatment, and plants were arranged randomly within the growth chamber. Treatments were replicated five times over time in the same growth chamber.

In a separate experiment, growth was assessed in field-grown Clark and Clark 63 plants by using a naturally occurring mutant of Xcg-8 resistant to the antibiotic rifampin. A single inoculum concentration was used (40 cfu/cm^2). Sampling methods were as described above except that the plating medium contained 50 μg per milliliter of rifampin (Sigma Chemical Co., St. Louis, MO). Leaves were sampled 0, 1, 4, 7, 10, 14, and 18 days after inoculation. Treatments were replicated five times. The experiment was conducted from 8 July 1982 to 26 July 1982. Temperature ranged from 25 to 35 C for daytime highs and 15 to 21 C for nighttime lows.

Infectivity titrations. Dose-response relationships were determined for three susceptible cultivars (Clark, Oakland, and BSR 301) and three resistant cultivars (Clark 63, Williams, and BSR 302) by counting the number of pustules per square centimeter of leaf area 10 days after inoculation. Pustules were counted with the aid of a stereoscopic microscope (10× magnification). Samples consisted of two microscopic fields per leaflet, three leaflets per plant on three plants. Initial inoculum concentrations (0, 0.6, 3.2, 16, and 80 cfu/cm^2) were determined as described above. Treatments were arranged in a randomized complete-block design with five replications. Slopes of regression lines were compared by analysis of variance (21).

Histological procedures. An initial inoculum concentration of 400 cfu/cm^2 was used for plants to be prepared for light and electron microscopy. This inoculum concentration resulted in confluent pustule development in the inoculated area. Tissues were harvested from Clark and L61-4180 plants 2 and 4 days after inoculation. Tissues from healthy control plants (infiltrated with sterile buffer) were harvested and processed along with the diseased material. Specimens were fixed for 6 hr at 22 C in 4% glutaraldehyde in 0.08 M phosphate buffer, pH 7. Tissue was then rinsed in buffer and postfixed overnight at 4 C in 1% osmium tetroxide in the same buffer. After dehydration in ethanol, material was transferred to propylene oxide and embedded in Spurr's resin. Sections were made with glass knives and stained with toluidine blue for light microscopy (LM) or uranyl acetate and lead citrate for electron microscopy (EM). Individual pustules were harvested from Clark and L61-4180 plants 10 days after inoculation with lower inoculum concentrations, which resulted in formation of scattered pustules within the inoculated area. These samples were prepared for microscopy as described above. In addition, 14-day-old pustules were collected from field-inoculated Clark and Clark 63 plants as well as from greenhouse-inoculated Harosoy and L61-4094 plants. These tissues were fixed and stored in FAA (50 ml of 95% ethanol, 5 ml of acetic acid, 10 ml of formalin, 35 ml of water) preceding freehand sectioning.

Morphometric methods. Tissues prepared for LM and EM were analyzed quantitatively by using standard stereological techniques (26). Morphometric analyses were carried out on Clark and L61-4180 leaf tissues collected 4 days after inoculation (or infiltration with sterile buffer). One tissue block was examined from each of four plants per treatment. Samples were examined at three levels of magnification: 450× and 1,000× (LM) and 18,000× (EM). At 450× and 1,000×, point counts were made by using a square lattice grid within the eyepiece of the microscope. At 18,000×, both point counts and line intersections were determined by using a test grid consisting of a staggered array of 1-cm lines, which was superimposed on the micrograph. Point and line intersection counts were used to generate two types of values: relative volumes and surface densities. Relative volume (or volume fraction) is that proportion of a volume occupied by a particular component of the volume. Surface density is a measure of interface surface area expressed as surface area per unit volume. At 450× and 1,000×, data were accumulated by using the entire tissue block face. At 18,000×, micrographs were taken of four randomly chosen palisade mesophyll cells and four randomly chosen spongy mesophyll cells per tissue block. Cells were chosen randomly by photographing one cell in each of four adjacent openings in the copper specimen grid. The cell photographed was that closest to the center of the grid opening. Volume fraction data (expressed as percentages) were subjected to arc sine transformation before analysis of variance (25). Means were separated by using an LSD test.

RESULTS

Pathogen population development. At all inoculum concentrations, populations of *X. c.* pv. *glycines* increased at similar rates in leaves of the susceptible cultivar Clark and the resistant Clark 63 (Fig. 1). The mean generation time, determined between 1 and 3 days after inoculation, was 10.49 hr. Symptoms in both cultivars developed more slowly at the lower inoculum concentrations, and abscission occurred only at the highest inoculum concentration. Pathogen growth in the cultivars Williams and BSR 301 (examined only at an initial inoculum concentration of 5 cfu/cm^2) was similar to that observed in Clark and Clark 63 (data not shown).

Populations of a rifampin-resistant mutant of *X. c.* pv. *glycines* developed at the same rate and reached the same stationary phase levels in Clark and Clark 63 plants inoculated in the field (Fig. 2). The average generation time was 10.43 hr.

Infectivity titrations. Dose-response relationships were determined by counting the number of pustules per square centimeter of leaf area 10 days after inoculation. For both the susceptible and resistant cultivars, the number of pustules was proportional to the inoculum concentration (Fig. 3). For the susceptible cultivar Clark, the slope of the regression line was 0.75, with a coefficient of determination (r) of 0.74. For Clark 63, the slope was 0.13 and $r = 0.56$. From this information, a lesion-forming unit (lfu) could be determined. An lfu is an estimate of the number of pathogen cells necessary to initiate a single lesion (5). For Clark, the lfu was 1.3 cfu, and for Clark 63, it was 8.2. The

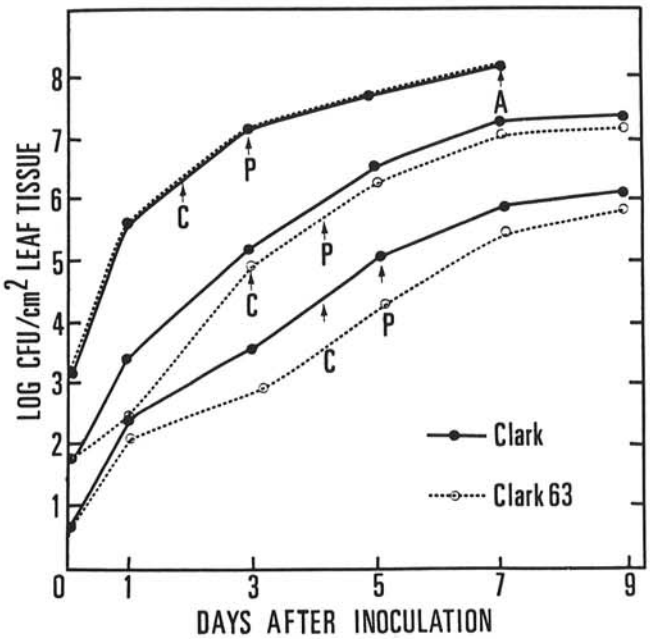


Fig. 1. Growth of *Xanthomonas campestris* pv. *glycines* in leaves of resistant (Clark 63) and susceptible (Clark) soybean cultivars. The three pairs of curves represent three levels of initial inoculum: 5, 70, and 1,600 cfu/cm^2. Arrows indicate time of symptom development (C = chlorosis, P = pustule formation, A = abscission).

other soybean cultivars tested responded in a similar fashion (Fig. 3) with lfu values as follows: BSR 301, 1.4; BSR 302, 15.9; Oakland, 1.4; Williams, 11.6. At initial inoculum concentrations in excess of 80 cfu/cm^2, confluent pustules developed on susceptible cultivars, and individual infection sites could not be counted.

Pustule development. Under the various conditions maintained in this investigation, pustules first became visible 3–5 days after inoculation and were fully developed 7–12 days after inoculation. Pustules were generally more easily observed on the undersides of the leaves. Individual pustules on field-inoculated Clark plants were 0.45 mm in diameter and 0.31 mm in thickness, whereas on Clark 63 plants, pustules were 0.34 mm in diameter and 0.23 mm in thickness. Healthy leaves were 0.16 mm thick. Pustules on greenhouse-inoculated Harosoy plants were 0.34 mm in diameter and 0.23 mm in thickness, whereas on L61-4094, pustules were 0.29 mm in diameter and 0.16 mm in thickness. Healthy leaf thickness was 0.13 mm. Pustules on the resistant cultivars (Clark 63 and L61-4094) were significantly smaller in both diameter and thickness than those on the corresponding susceptible cultivars (Clark and Harosoy) ($P = 0.05$).

Histological observations and morphometric analyses. The near-isogenic lines Clark (susceptible) and L61-4180 (resistant) were used in these studies. Inoculated plants used in morphometric analyses received an inoculum concentration sufficient to result in confluent pustule development. Nonvascular tissues in a healthy soybean leaf (Fig. 4) consist of upper and lower epidermis, palisade and spongy mesophyll, and a layer of stellate cells located between the palisade and spongy mesophyll called the paraveinal mesophyll (15). Inoculated leaves typical of those used in morphometric studies are shown in Figures 5 and 6. In these tissues, collected 4 days after inoculation, necrotic cells were rarely observed. Although hypertrophy was not yet fully expressed, leaves were significantly thicker in the inoculated plants (196 μm vs. 147 μm for Clark, 177 μm vs. 135 μm for L61-4180). ($P = 0.05$). All nonvascular tissues could undergo hypertrophy; however, the response was first observed and generally developed to the greatest extent in the spongy mesophyll. The relative volumes of tissues in healthy and diseased leaflets are presented in Table 1. The proportional volume occupied by the spongy mesophyll increased in the diseased plants at the expense of the volume occupied by the intercellular space. In discrete pustules obtained from leaves receiving lower inoculum concentrations, these same general trends could be seen (Figs. 7 and 8). Pustules resulted solely from hypertrophy of cells in infected leaves; hyperplasia was never observed.

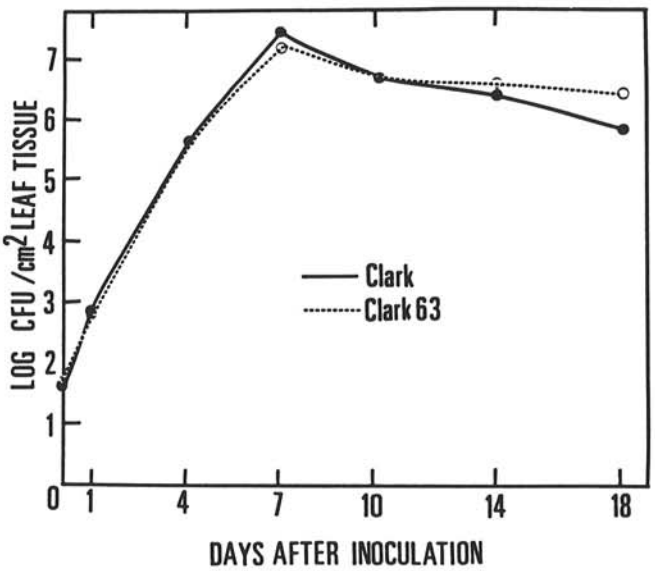


Fig. 2. Growth of a rifampin-resistant strain of *Xanthomonas campestris* pv. *glycines* in leaves of field-grown soybeans of the cultivars Clark (susceptible) and Clark 63 (resistant). Initial inocululm concentration was 40 cfu/cm^2.

Data concerning cellular organization in healthy and diseased mesophyll tissues are presented in Tables 2–4. Mesophyll cells typical of those used in collection of morphometric data are illustrated (Figs. 9–12). By 4 days after inoculation, the absolute volume of palisade mesophyll cells had increased 33% in infected Clark leaflets and 24% in infected L61-4180 leaflets. Spongy mesophyll cells had increased in volume 194% in infected Clark leaflets and 179% in infected L61-4180 leaflets. Most of the volume increase could be accounted for by the increase in vacuolar volume (Table 2). Within the cytoplasm of the palisade and spongy mesophyll cells, an increase in the relative volume of the hyaloplasm (ground cytoplasm) in infected leaflets was accompanied by a decrease in the relative volume of plastids (Table 3). The most dramatic change noted in the diseased tissues was the increase in the amount of endoplasmic reticulum (Table 4, Fig. 12). Plastids in infected mesophyll tissues had less starch and more numerous plastoglobuli than those in healthy tissues. Paracrystalline arrays of phytoferritin (Fig. 11), which were rarely seen in healthy leaves, were quite common in the infected tissues.

DISCUSSION

Our goal in this work has been to characterize bacterial pustule resistance conditioned by the *rxp* gene. This resistance seems to be very unusual. Under natural conditions in the field, plants carrying the *rxp* gene rarely show any symptoms. However, after inoculation, pathogen growth is unrestricted within the leaves of resistant cultivars. Even though pathogen growth is unrestricted, fewer pustules develop on leaves of resistant cultivars, and those that do form are somewhat smaller than those developing on susceptible cultivars. This reduction in pustule frequency probably is an important aspect of bacterial pustule resistance.

The growth of bacterial pathogens in highly resistant cultivars is generally impeded relative to the growth observed in susceptible cultivars (8,11,12,24). In cases where pathogen populations reach similar levels in resistant and susceptible cultivars, it is usually due to the use of artificially high inoculum concentrations (8) or introduction of bacteria into tissues where resistance is not expressed (3). However, our findings indicate that the bacterial pustule pathogen grows equally well in susceptible and highly

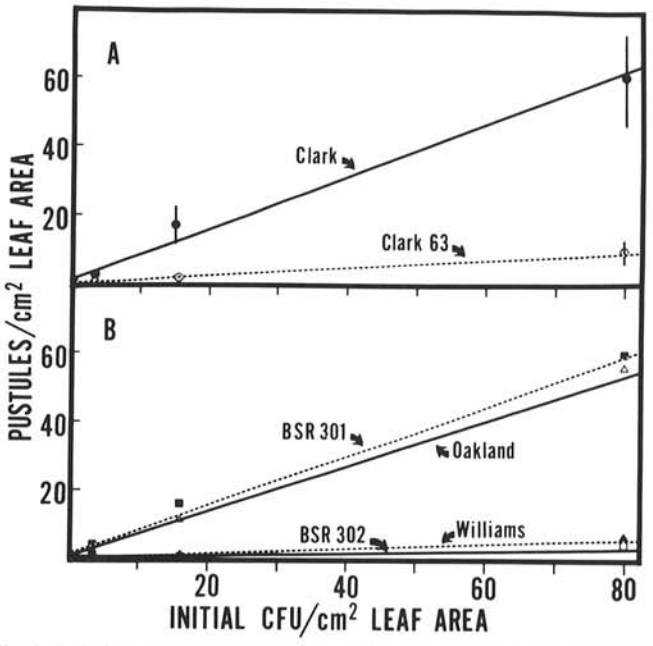


Fig. 3. Relationship between inoculum concentration and the number of pustules formed 10 days after inoculation. Recently expanded first trifoliolate leaves were inoculated with *Xanthomonas campestris* pv. *glycines* by using an airbrush. **A,** Susceptible cultivar Clark and resistant Clark 63. Bars indicate standard errors. **B,** Susceptible cultivars BSR 301 and Oakland, resistant cultivars BSR 302 and Williams.

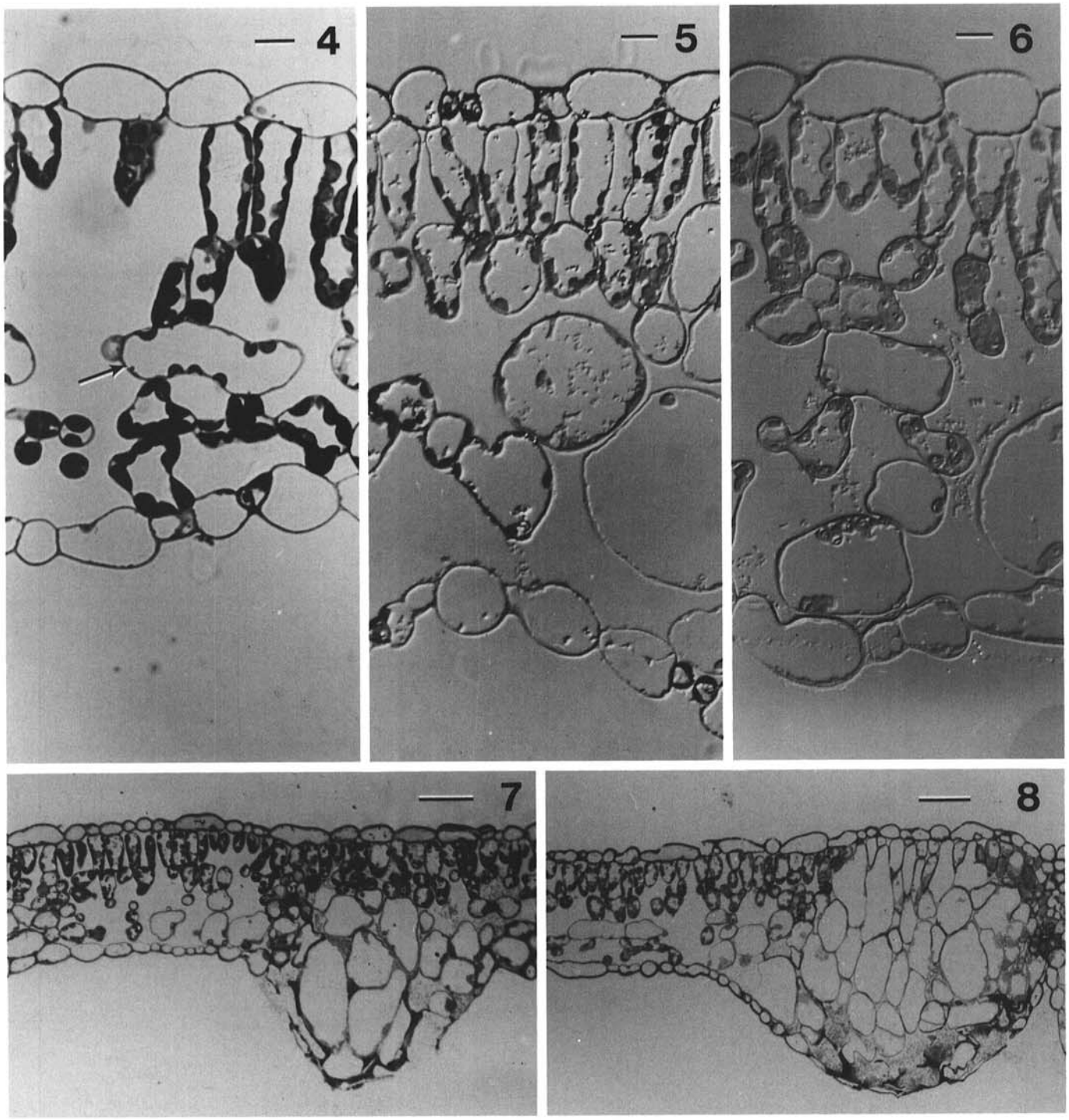
TABLE 1. The relative volume of nonvascular tissues in healthy soybean leaflets and those infected with *Xanthomonas campestris* pv. *glycines*[a]

	Upper epidermis	Palisade mesophyll	Paraveinal mesophyll	Spongy mesophyll	Lower epidermis	Intercellular space
Clark control[b]	11.4 a[c]	27.5 a	6.3 a	8.0 a	8.8 a	38.0 a
Clark diseased	8.0 b	27.5 a	7.3 a	21.3 b	8.5 a	26.3 b
L61-4180 diseased	8.3 b	26.5 a	5.5 a	18.5 b	11.3 b	30.0 b
L61-4180 control	9.5 ab	28.1 a	6.3 a	8.7 a	9.7 ab	37.9 a

[a] Plants sampled for microscopy 96 hr after airbrush inoculation. Initial population density of 400 cfu/cm^2 of leaf tissue. Control plants infiltrated with sterile 0.01 M phosphate buffer, pH 7.

[b] Clark and L61-4180 are near-isogenic lines, with L61-4180 resistant to bacterial pustule.

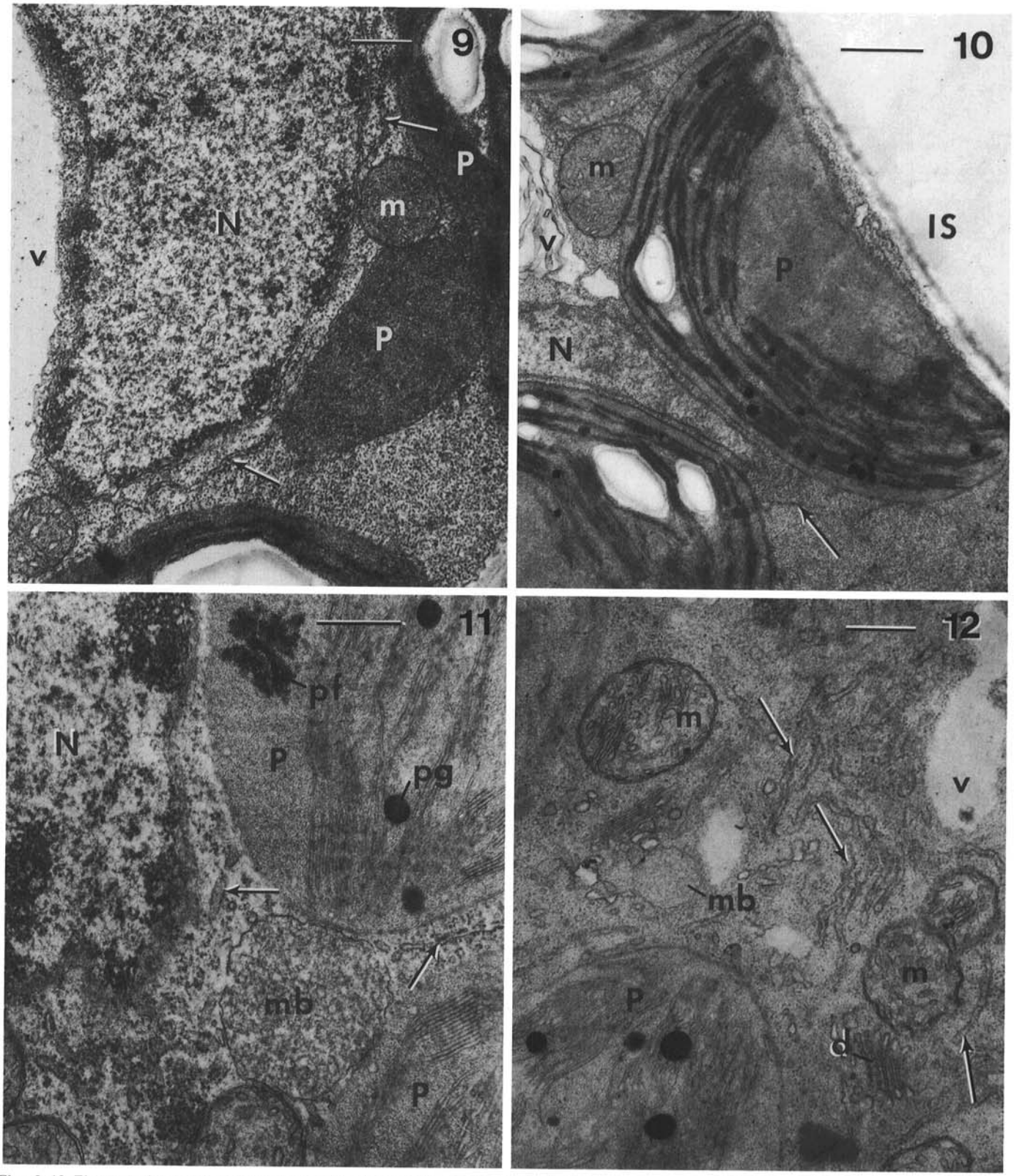
[c] Numbers in column followed by a common letter not significantly different ($P = 0.05$). Relative volumes reported as percentages.



Figs. 4–8. Light micrographs of healthy and diseased soybean leaves. **4,** Healthy soybean leaf, cv. Clark. Arrow indicates cell of paraveinal mesophyll tissue. Bright field. Bar = 10 μm. **5,** Infected leaf, cv. Clark, 4 days after inoculation. Hypertrophy is most evident in spongy mesophyll tissue. Interference contrast. Bar = 10 μm. **6,** Infected leaf, cv. L61-4180, 4 days after inoculation. Small objects in intercellular spaces are bacteria. Interference contrast. Bar = 10 μm. **7 and 8,** Fully developed pustules, cv. Clark, 10 days after inoculation. Bright field. Bars = 50 μm.

resistant soybean cultivars even at very low inoculum levels. These results agree with those recently reported by Fett using a different strain of *X. c.* pv. *glycines* (14). In contrast, Chamberlain (4) stated that populations of *X. c.* pv. *glycines* developed at a slower rate in the resistant cultivar CNS than in the susceptible cultivar Lincoln. Inspection of his data, however, indicates that, except for a short lag period (2 days) in CNS, the populations in both cultivars increased at the same rate, and at 25 days after inoculation, the total populations were about equal.

The reduction in pustule frequency observed with the resistant cultivars is due to an increase in the number of pathogen cells needed to initiate pustule formation. Under our experimental conditions, it took approximately 1.3 cfu to initiate a pustule in the susceptible cultivar Clark and 8.2 cfu in the resistant cultivar Clark



Figs. 9–12. Electron micrographs of healthy and diseased soybean mesophyll cells. **9,** Healthy spongy mesophyll cell, cv. Clark. Bar = 1 μm. **10,** Healthy spongy mesophyll cell, cv. Clark. Bar = 1 μm. **11,** Palisade mesophyll cell in infected leaf, cv. Clark. Bar = 0.5 μm. **12,** Palisade mesophyll cell in infected leaf, cv. L61-4180. Bar = 0.5 μm. Legend: N = nuclei; P = plastids; m = mitochondria; mb = microbodies; d = dictyosomes; v = vacuoles; pg = plastoglobuli; pf = phytoferritin. Arrows denote endoplamsic reticulum.

TABLE 2. The relative volumes of nuclei, cytoplasm, and vacuoles in the mesophyll of healthy soybean leaflets and those infected with *Xanthomonas campestris* pv. *glycines*[a]

	Palisade mesophyll			Spongy mesophyll		
	Nuclei	Cytoplasm	Vacuoles	Nuclei	Cytoplasm	Vacuoles
Clark control[b]	3.5 a[c]	52.8 ab	43.5 ac	3.8 a	55.0 a	41.3 a
Clark diseased	3.8 a	38.3 c	58.0 b	1.3 c	25.0 b	73.3 b
L61-4180 diseased	3.5 a	42.5 bc	53.3 ab	2.3 bc	27.8 b	69.5 b
L61-4180 control	3.7 a	54.6 a	39.2 c	3.2 ab	59.2 a	37.6 a

[a] Plants sampled for microscopy 96 hr after airbrush inoculation at initial population density of 400 cfu/cm^2 of leaf tissue. Control plants were infiltrated with sterile 0.01 M phosphate buffer, pH 7.
[b] Clark and L61-4180 are near-isogenic lines, with L61-4180 resistant to bacterial pustule disease.
[c] Numbers in column followed by a common letter not significantly different ($P = 0.05$). Relative volumes reported as percentages.

TABLE 3. The relative volume of cytoplasmic components in the mesophyll of healthy soybean leaflets and those infected with *Xanthomonas campestris* pv. *glycines*[a]

	Plastids	Mitochondria	Microbodies	Dictyosomes	Lipid bodies	Hyaloplasm
Palisade mesophyll						
Clark control[b]	72.0 a[c]	4.1 a	0.6 a	0.4 a	0.0 a	22.6 a
Clark diseased	54.1 b	5.8 b	1.5 b	1.5 b	0.7 b	36.1 b
L61-4180 diseased	62.8 c	6.1 b	2.2 b	0.5 a	0.0 a	28.7 c
L61-4180 control	69.9 a	6.2 b	2.3 b	0.4 a	0.1 a	21.3 a
Spongy mesophyll						
Clark control	70.6 x	5.1 x	1.3 x	0.4 x	0.0 x	22.8 x
Clark diseased	41.4 y	8.1 y	0.6 x	0.8 x	1.1 y	48.0 y
L61-4180 diseased	51.1 y	5.1 x	1.6 x	1.6 x	0.6 z	40.8 y
L61-4180 control	73.6 x	3.9 x	1.0 x	0.7 x	0.0 x	20.9 x

[a] Plants sampled for microscopy 96 hr after airbrush inoculation. Initial population density of 400 cfu/cm^2 of leaf tissue. Control plants infiltrated with sterile 0.01 M phosphate buffer, pH 7.
[b] Clark and L61-4180 are near-isogenic lines, with L61-4180 resistant to bacterial pustule.
[c] Numbers in column followed by a common letter are not significantly different ($P = 0.05$). Relative volumes reported as percentages.

63. If we assume that pathogen cells act independently in eliciting a host response (as has been demonstrated for several phytopathogenic bacteria [5,11,12]), then the probability that a given cell would initiate an infection was approximately 0.75 in the susceptible cultivars and 0.13 in the resistant cultivars. The young leaves that we inoculated and the favorable environment maintained in our greenhouse and growth-chamber studies probably constitute nearly ideal conditions for bacterial pustule development. We expect that the infection efficiency would be lower under natural conditions in the field.

The anatomical responses to infection were identical in both the resistant cultivar L61-4180 and the susceptible cultivar Clark, although the responses were of somewhat reduced intensity in L61-4180. This was true whether the responses were measured at the whole-tissue level or at the subcellular level.

Our observation that pustules arise solely through hypertrophy agrees with that of Wolf (28). In fully developed pustules, it is not uncommon to find mesophyll cells that are 10 times larger than those in healthy leaves. The increase in cell volume in infected tissues can be accounted for primarily by an increase in the volume of the vacuole. Cell expansion is thought to result from water uptake into the vacuole after an increase in cell wall plasticity (27). Although auxin is known to increase the plasticity of cell walls (27) and *X. c.* pv. *glycines* is known to be capable of producing auxin in culture (22), the role played by this hormone in pustule development has not been determined. Expansion growth should not be interpreted as simple passive water uptake, however, because the increase in cell size is accompanied at the very least by active synthesis of cell membranes and cell walls (the walls do not become thinner as the cell expands). It was not surprising to find a very large increase in the surface density of ER in cells undergoing hypertrophy inasmuch as this organelle is known to play an important role in the synthesis of membrane proteins and phospholipids as well as protein and matrix materials involved in cell wall synthesis (18,23).

The hypertrophic mesophyll cells of the type seen 4 days after inoculation have some features in common with cells in early stages of senescence (1,15,18,20). Lipid bodies, which are rare in the cytoplasm of healthy cells, can be easily found in infected tissues. Plastids contain abundant plastoglobuli and accumulations of phytoferritin, although their starch is depleted. These hypertrophied cells appear to remain viable (based on general appearance and organization of the cytoplasm) for several days while presumably supplying the intercellular pathogen populations with nutrients. Cell death does not seem to occur until the later stages of pustule development. It is our impression that necrosis occurs rapidly because cells in intermediate stages of collapse are rarely seen.

TABLE 4. Surface density[a] of endoplasmic reticulum in mesophyll cells of healthy soybean leaflets and those infected with *Xanthomonas campestris* pv. *glycines*

	Palisade mesophyll	Spongy mesophyll
Clark control	0.81 a[b]	0.66 a
Clark diseased	1.65 b	1.85 b
L61-4180 diseased	1.17 c	1.44 c
L61-4180 control	0.47 d	0.45 a

[a] Expressed in $\mu m^2/\mu m^3$.
[b] Numbers in column followed by a common letter are not significantly different ($P = 0.05$).

When resistant plants carrying the *rxp* gene are inoculated with *X. c.* pv. *glycines* in the greenhouse, growth chamber, or field, they do become diseased, although fewer and smaller pustules develop compared with susceptible plants. Under field conditions, where resistant cultivars are planted over large areas away from susceptible plants and no inoculum is applied, they remain symptomless. We have observed that the epiphytic populations of *X. c.* pv. *glycines* that develop on susceptible cultivars in the field are much larger than those developing on resistant cultivars (17). Similar observations have been made with several other bacterial diseases (7,9). Crosse (6,7) felt that resistance of certain cherry

cultivars to *Pseudomonas mors-prunorum* was due to a reduced inoculum potential (smaller epiphytic populations on the leaf surface) coupled with a reduced infection efficiency (greater inoculum concentration necessary for infection). We believe that bacterial pustule resistance may operate in a similar fashion.

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