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The hypersensitive reaction of cotton to *Xanthomonas campestris* pv. *malvacearum*

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Bacterial blight of cotton (*Gossypium* spp.) incited by *Xanthomonas campestris* pathovar *malvacearum* (Smith) Dye (*Xcm*) occurs in most producing regions of the world. This seed-borne disease, potentially very destructive, can affect all stages and parts of the cotton plant throughout the growing season. The disease has been successfully controlled using resistant varieties. At least 19 genes conferring resistance to bacterial blight (B-genes) have been identified (25, 37). In Africa, the resistance (immunity) to the bacterial blight pathogen in commercial cultivars has been attributed to the combinations of two major resistance genes (*B_2B_3* or *B_2B_6*).

The permanent threat from the disease mainly results from genetic variability of the pathogen which can nullify the effects of resistance genes in the field (7, 8). On the basis of disease reactions of a set of Upland cotton differentials (3, 26), 20 races have been identified (20, 25). The investigations by Gabriel and co-workers (13, 14, 21, 22) led to the conclusions that the genetics of race-cultivar specificity in *Xcm* on cotton exhibit gene-for-gene as well as gene-for genes pattern of interaction. Such interactions are usually defined in term of compatible (normosensitive reaction i.e. water-soaked lesions) and of incompatible interactions (disease resistance) characterized by a rapid, localized cell death at sites of infection, a phenomenon known as the hypersensitive reaction (HR) (23, 27). This reaction is considered to be an important component of resistance of cotton to bacterial blight (17, 21). In this paper we present a brief survey of the incompatible interaction with emphasis of virulence diversity and the HR.

I Hypersensitive reaction and bacterial growth

Resistance of cotton to *Xcm* is generally associated with the rapid induction of a HR (17, 21). The HR is macroscopically observed as confluent lesions when cotyledons or leaves are infiltrated with high titers of bacterial suspension (about 10^7 colony forming units or more per ml). This reaction becomes visible in cotton leaves within 8-12 hours post-inoculation (h.p.i.) (Fig 1A). However, the phenotype of HR on cotton differs according to race of *Xcm* and the complementary resistance genes in the host examined (13, 21). These phenotypes are distinguished by the timing of the onset of cell collapse, the intensity of discoloration, and the degree of confluence.

Under field levels of inoculum (10^6 cfu/leaf or cotyledon, or lower), host mesophyll cells react hypersensitively 3 or 4 days after inoculation (21). According to the pair of interacting race and resistant cultivar, the plants remained macroscopically symptomless or showed small, dry, round-to-angular leaf lesions (8). In experiments during which cotton leaves were spray-inoculated with a bacterial suspension of $5x10^6$ cfu/ml (Fig. 2), bacterial growth

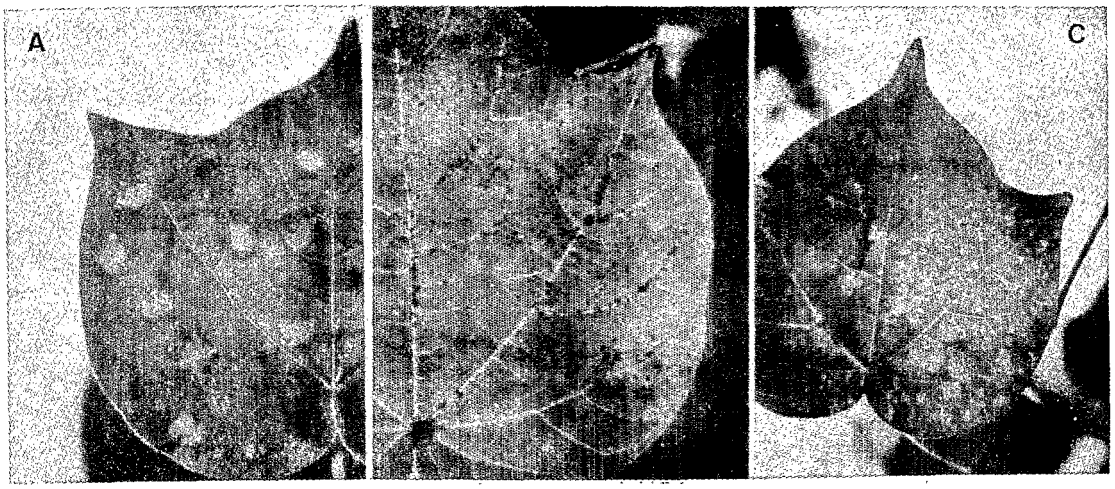
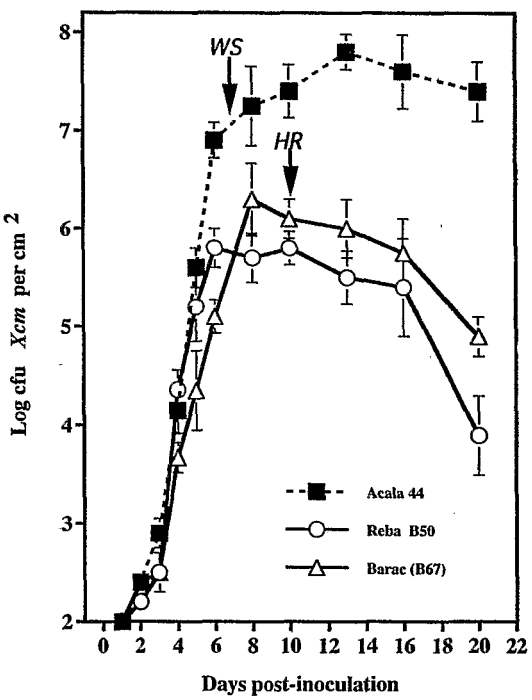


Figure 1. Bacterial blight reactions on resistant and susceptible cotton lines following leaf inoculation of *Xanthomonas campestris* pathovar *malvacearum*.
A. Hypersensitive reactions following leaf infiltration of the resistant cultivar Reba B50 with a suspension of 10^8 cfu/ml.
B. Localized hypersensitive responses following spray inoculation of the resistant cultivar Barac (67)B with a suspension of 5×10^6 cfu/ml.
C. Compatible reaction (angular, water-soaked spots) on the susceptible cultivar Acala 44 following spray inoculation with a suspension of 5×10^6 cfu/ml.



was logarithmic for 6 days in the susceptible Acala 44 and the resistant Réba B50 and Barac (67)B, but stopped at lower population densities in resistant leaves ($5x10^5$ to $2x10^6$ bacteria/cm^2). The bacterial growth curves (Fig. 2) indicate that pathogen growth inhibition occurred 2 days earlier in Reba B50 than in Barac (67)B. Small and dry HR lesions developed (days 9) on Barac 67B leaves (Fig. 1B), while Reba B50 remained macroscopically symptomless. In the susceptible Acala 44, growth of *Xcm* continues to reach a final population ranging from about 10^7 to 10^8 cfu/cm^2. Angular water-soaked lesions were visible by 7 to 8 days after inoculation (Fig. 2C). After this

Figure 2. Growth kinetics of *Xanthomonas campestris* pathovar *malvacearum* in spray-inoculated leaves of susceptible Acala 44 and resistant Reba B50 and Barac(67)B cotton lines.
WS = water-soaked lesions; HR = localized macroscopic hypersensitive reaction.

period, no further growth was visible. In resistant lines, bacterial populations remained confined to the infection site resulting from the localized HR cell death, and declined.

II Genes controlling the hypersensitive reaction in cotton

Race specific resistance to *Xcm* depends on the presence of two different loci: a resistance locus in a particular line of cotton and an avirulence locus in a particular race of *Xcm* (21). From analysis of avirulence in *Xcm*, 12 *avr* genes have been cloned and characterized (9, 14). These genes belong to a large family of xanthomonads *avr* (avirulence)/*pth* (pathogenicity) genes that comprise nearly all of *Xanthomonas* avr genes cloned to date. Six of the *avr* genes are plasmid-borne (*avrB4, avrb6, avrb7, avrBIn, avrB101, avrB102*), while the others appear to be located on the bacterial chromosome. The avirulence specificities of these genes were shown to be determined by the presence of the 102 bp tandem repeats in the central region of the genes (40). Interestingly, some members of the *Xcm avr/pth* genes (*avrb6, avrb7, pthN, pthN2*) were found to pleiotropically encode avirulence on resistant host and pathogenicity by contributing to water-soaking symptoms on susceptible cotton plants (40, 41).

Analysis of the avirulence genes indicated that they are necessary but not sufficient to elicit HR. The ability of *avr* genes in the elicitation of the HR is dependent on functional hypersensitive reaction and pathogenicity (*hrp*) genes (30). Growing evidences demonstrate that these genes are clustered and highly conserved among different phytopathogenic xanthomonads (6). As determined by Southern hybridization experiments homologous DNA sequences to *hrp* genes have been detected in *Xcm* (5, 29). These observations were extended by *hrp*-PCR using primers based on *hrp* sequences of *X. c.* pv. *vesicatoria* (29). This method allowed to amplify two *hrp* fragments of the expected length (830-bp; 1,075-bp) from total genomic DNA of differents strains of *Xcm* (Fig. 3). Whether any of these homologies between *hrp* regions of *X. C.* pv. *vesicatoria* and *Xcm* are related to homologous functions remains to be determined.

Mutations of *hrp* genes result in changes in HR and/or pathogenic ability (30) while mutations of *avr* genes alone affect cultivar-specific avirulence and in some cases species-specific pathogenic functions as water soaking (14, 39). It is interesting to note that

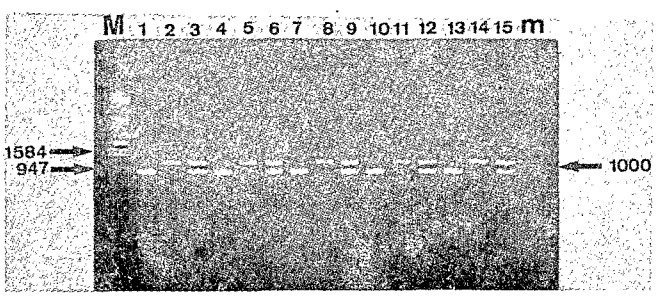


Figure 3. Amplification of two fragments of the *hrp* gene cluster from 5 strains of *Xanthomonas campestris* pathovar *malvacearum*.
Lanes 1, 4, 7, 10, 13 : amplification of the 840-bp fragment with primer pairs RST2 and RST3; lanes 2, 5, 8, 11, 14 : amplification of the 1075-bp fragments with primer pairs RST21 and RST22; lanes 3, 6, 9, 12, 15, multiplex amplification of the 840-bp and 1075-bp fragments with two primer pairs (RST2-RST3 and RST21-RST22). Lanes: M, phage restricted with *Eco*RI and *Hind* III; m, 100 -bp DNA ladder. Molecular sizes are given in base pairs.

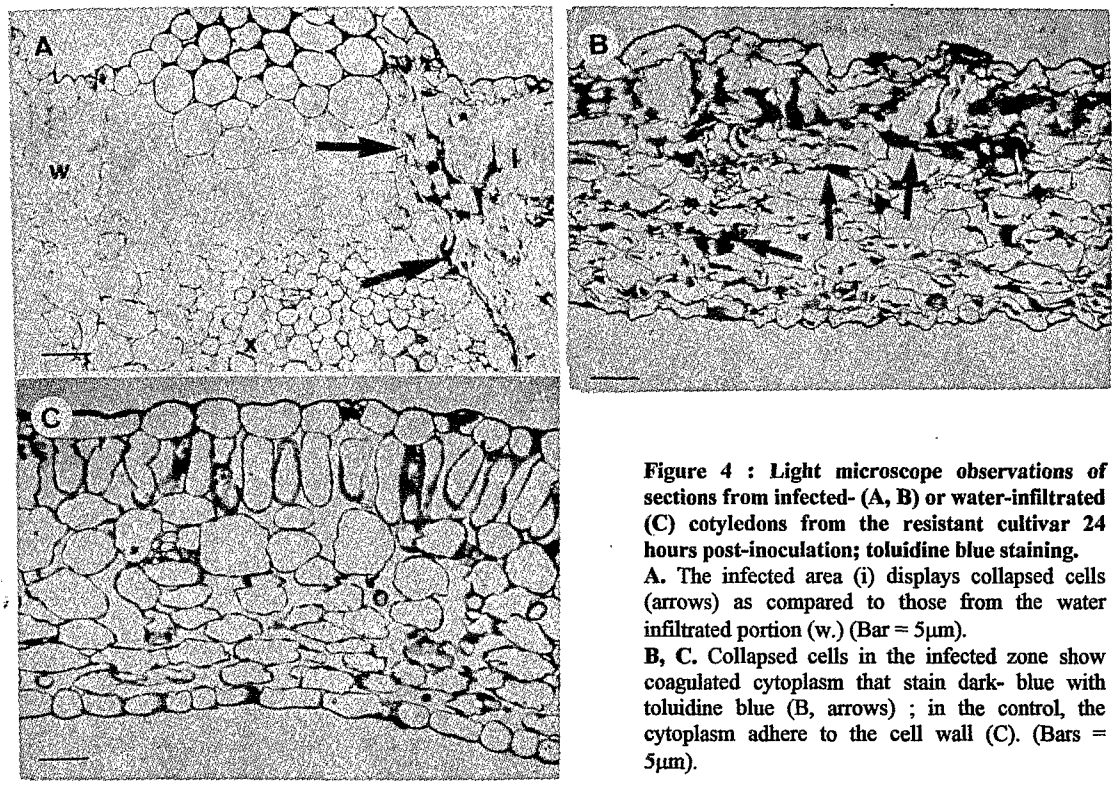


Figure 4 : Light microscope observations of sections from infected- (A, B) or water-infiltrated (C) cotyledons from the resistant cultivar 24 hours post-inoculation; toluidine blue staining.
A. The infected area (i) displays collapsed cells (arrows) as compared to those from the water infiltrated portion (w.) (Bar = 5μm).
B, C. Collapsed cells in the infected zone show coagulated cytoplasm that stain dark- blue with toluidine blue (B, arrows) ; in the control, the cytoplasm adhere to the cell wall (C). (Bars = 5μm).

intragenic recombination in a *avr/pth* gene can result in loss of pathogenic phenotypes, but generates new avirulence specificities indicating that disease reactions and HR may share similar signal transduction pathway, as initially suggested by Klement (27).

III Physiology of the hypersensitive response to cotton

The HR is a response of plants elicited by pathogenic microbes during incompatible or non-host interactions. Symptoms appear as lesions, the cells of which are characterized by an extremely rapid death, aiming at limiting the progression of the infection. Cotton cotyledons differentiated HR within 24 h.p.i., dessication and dryness of the infected tissues occurring 72 h.p.i.

A. Ultrastructural and histological aspects of HR

In the resistant line Reba B50, modifications of infected tissues were visible 6 h.p.i. at sites where the inoculum was infiltrated, as compared to water-infiltrated cotyledons. Ten to 24 h.p.i., light microscopy revealed degraded cells in the infected portion of cotyledons (Fig. 4A), while tissues adjacent to the necrosis showed no apparent modifications. Cells in the necrotic area (Fig. 4B) exhibited intensily stained cytoplasm after using toluidine blue. Seventy-two h.p.i., the necrotic areas of infected cotyledons became dry while the healthy portions did not. Infiltration of the resistant cotyledons with sterile water did not induce the formation of necrotic areas (Fig. 4C). In the susceptible line, Acala 44, rapid degradation of the infected tissues did not occur.

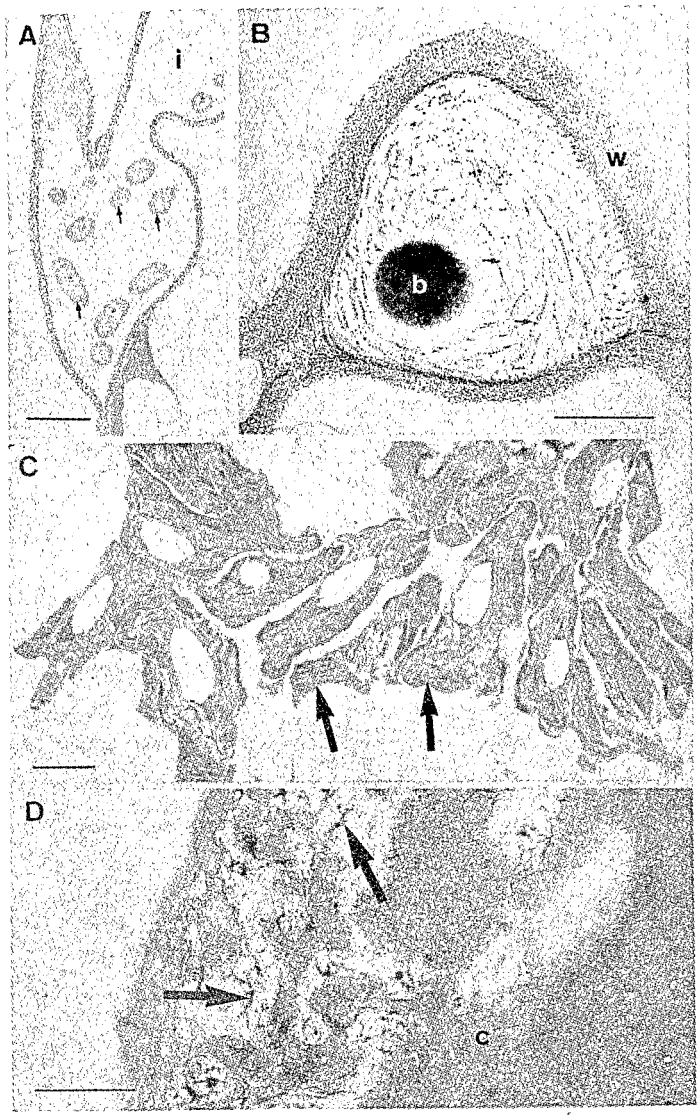


Figure 5: Ultrastructural observations of infected resistant cotyledons 24 hours post-inoculation; uranyl acetate and lead citrate staining.

A. In the infected areas, bacteria (arrows) are located intercellularly (i). (Bar = 1μm).

B. This bacteria (b) is surrounded by fibrillar exopolysaccharides (arrows) that closely adhere to the host cell wall (w). (Bar = 0.3μm).

C. The cytoplasm of cells that undergo the HR appear highly coagulated (arrows). (Bar = 1μm).

D. A fibrillar material occurs within the coagulated cytoplasm of HR cells (arrows). (Bar = 0.3μm).

Rather, water-soaked lesions developed 5-8 days after infection causing alterations of the tissues.

Ultrastructural observations showed that 20 h.p.i. in the resistant line, bacteria cells were located intercellularly (Fig. 5A) showing close association of exopolysaccharides with host cell walls (Fig. 5B). Mesophyll and phloem cells of veins located close to the necrotic area displayed various ultrastructural aspects, including separation of the plasmalemma from the cell surface associated with an increase in the electron-density of the cytoplasm. The infected mesophyll cells within the necrotic lesion showed a retracted and coagulated cytoplasm (Fig. 5C) containing fibrillar-like components (Fig. 5D). Important papillae were sometimes seen causing abnormal loss of cell shape (Fig. 6A). Such paramural deposits positiveley react to callose (Fig. 6B), but not to other plant polysaccharides probes, including cellulose and pectin. Intensely electron-dense bodies were found in this coagulated cytoplasm and in paramural areas (Figs. 6A, C). The middle lamella of electron-dense bodies-containing mesophyll cells was highly electron-dense. The fibrillar bacterial sheath of the pathogen located in intercellular spaces of the necrotic tissues was closely associated with the electron-dense middle lamella, fragments of which were seen to be enclosed within the bacterial sheath (Fig. 6D), or in close contact with it. Observations of HR lesions at a distance from the margin revealed that degraded cells contained residual cytoplasmic fragments. In sections from healthy tissues from water-infiltrated cotyledons, mesophyll cells did not display any electron-dense areas. In the infected susceptible line, disorganization of mesophyll cells consisted in a membrane alteration of organelles, including nuclei and chloroplasts (Fig. 7A).

B. The oxidative burst

It is now established that the "oxidative burst" is an important feature of plant resistance responses towards invading pathogens (1). The rapid production of active oxygen species (AOS) occurs at the early stage

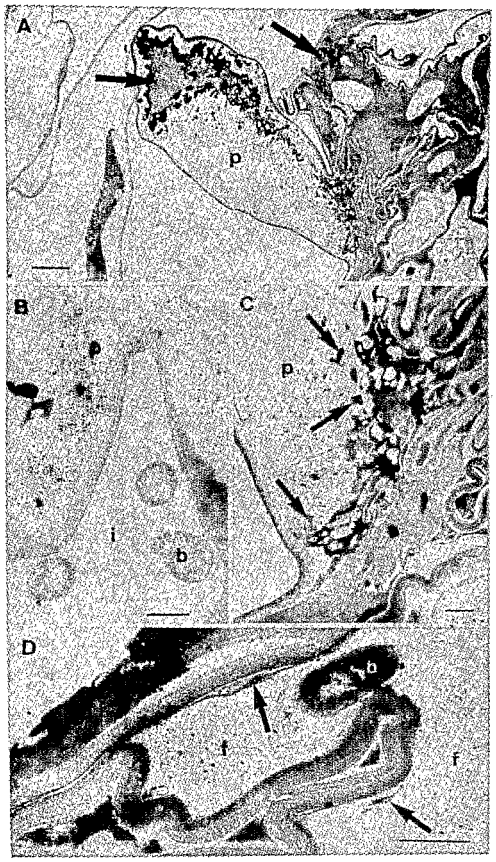


Figure 6 : Ultrastructural observations of infected resistant cotyledons 24 hours post-inoculation; uranyl acetate and lead citrate staining

A. A large papilla (p) is formed in the periplasmic area, resulting in a loss of cell shape and reduction of the coagulated cytoplasm (arrows). (Bar = 1.25μm).

B. Immunolocalization of callose using anti ß-1,3-glucans polyclonal antibodies. The papilla (p) is immunoreactive, showing an even distribution of gold particles. The bacteria is located (b) in the intercellular area (i). (Bar = 0.4μm).

C. Electron-dense bodies (arrows) accumulated in paramural papillae (p) and the coagulated cytoplasm. (Bar = 0.5μm).

D. The bacterial fibrillar material (f) is closely associated with host middle lamella and contains electron-dense fragments of host cell walls (arrows). (Bar = 0.3μm)

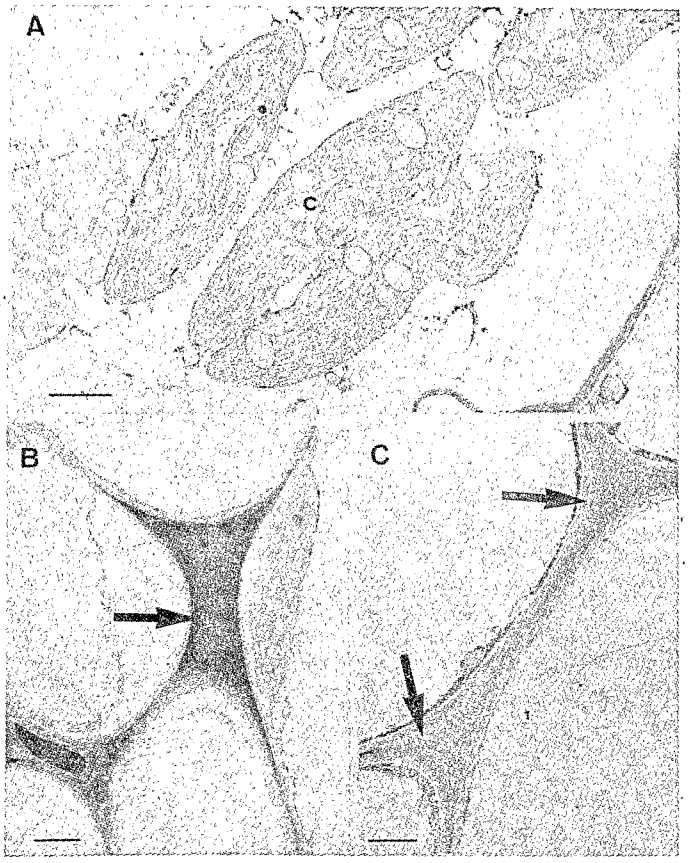


Figure 7 : Ultrastructural observations of infected susceptible cotyledons 24 hours post-inoculation (A); cytochemical localization of DAB-peroxidase activity 3 hours post-infection (B, C); uranyl acetate and lead citrate staining

A. In susceptible cotyledons , the cytoplasm of cells in the infected areas does not display any coagulated aspect, but shows important desorganization of membranes, including those of chloroplasts (c). (Bar = 1μm).

B,C. In the infected resistant cotyledons, the apoplast is highly electron-dense indicating the localization of peroxidase activity (B, arrow), as compared to the apoplast in the susceptible infected cotyledons (C, arrows) which does not show any significant activity. (Bars = 1μm).

of the HR (32). The production of AOS - free radicals - during interactions of *Xcm* race 18 with cottons cultivars has been assessed biochemically and histochemically. Between 2 and 3 h.p.i. with *Xcm* race 18, infected cotyledons from Reba B50 plants were characterized by a sharp burst of $O_2^{\cdot-}$, the production of which was inhibited by SOD. The use of peroxydase inhibitors (SHAM or KCN) demonstrated that superoxide anions resulted from the activation of a putative peroxidase (Fig. 8A). Inoculation of Reba B50 with either the virulent race 20 (compatible interaction) or heat-killed *Xcm* race 18 did not

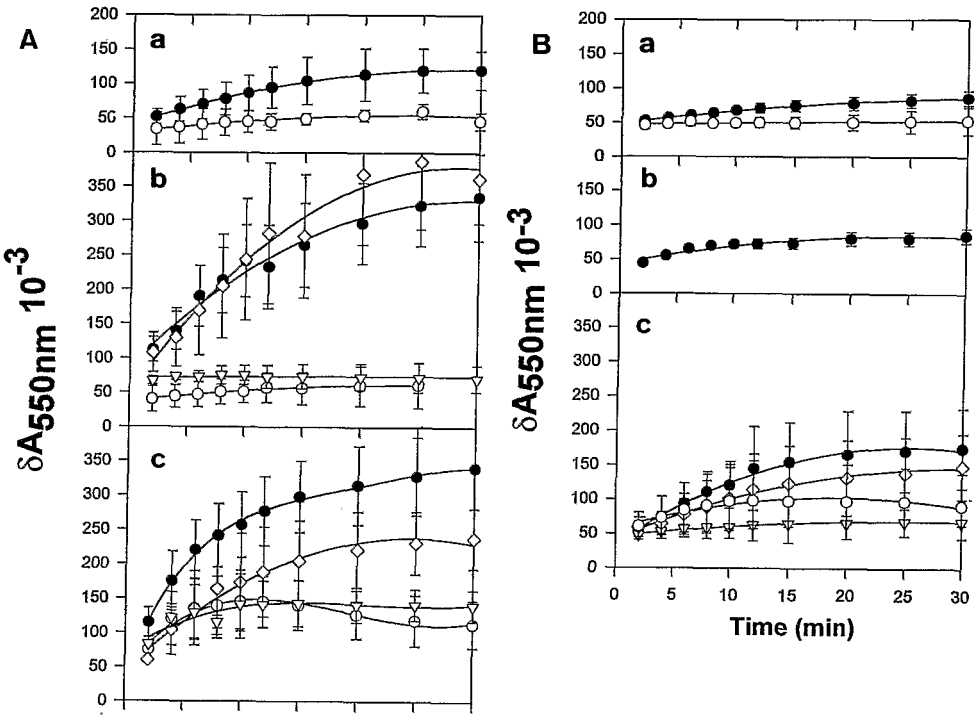


Figure 8: Production of reactive oxygen species.

A. Time course of extracellular cytochrome c reducing activity of Reba B50 3 h after infiltration with water (a, ●), with Xam race 18 (b, ●) or immediately after infiltration with 80 µM digitonin (c, ●). Absorbance at 550 nm of samples were read against a reference cuvette containing 20 µM cytochrome c+100 µMDFO in PBS. Reducing activities were monitored in presence of 1000 U/ml of SOD (○) or 20 µM DPI (◊) or 50 µM KCN (Δ). Values are means ± SD of 3 separate experiments with 5 replicates each.

B. Time course of extracellular cytochrome c reducing activity in cotyledons of the susceptible cultivar. Inoculations, treatments and number of replicates were identical as those in figure 8A.

resulted in the development of any $O_2^{\cdot -}$ burst. In constrast, infected cotyledons of the variety Acala 44 were unable to elicit any oxidative burst (Fig. 8B).

The time course evolution in the NADH oxidase activity in crude enzymatic extracts prepared from cotyledons inoculated with *Xcm* race 18, correlated that of the AOS production (Figs. 9A,B). This NADH oxidase activity was blocked by the peroxidase inhibitors, KCN and SHAM, but strongly stimulated by Mn^{2+} ions or 2,4-DCP, which were both described as powerful stimulating agents of peroxidase (24). The higher specific activity of NADH oxidation in apoplastic washing fluid preparations than that assessed in the crude extracts strongly suggests that this activity in cotton was likely localized in the apoplastic compartment.

C. Activation of defense genes

1. Flavonoid acculumation

Treatment of sections in cotyledons of the

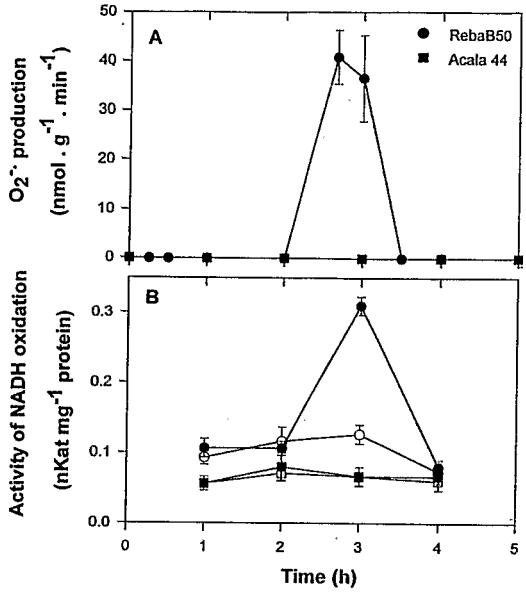


Figure 9 : Correlation between $O_2^{\cdot-}$ production and the NADH oxydase activity.

A. Time course changes in $O_2^{\cdot-}$ production; values of inoculated plants were substracted from the corresponding water infiltrated controls.
B. activity of NADH oxidation of crude enzymatic extracts of Reba B50 (●, ○) and Acala 44 (■, □) inoculated with Xam, race 18. $O_2^{\cdot-}$ production; open symbols correspond to NADH oxidation activity of water controls. Values are means ± SD of 2 separate experiments with 5 replicates each.

resistant line with the Neu's reagent revealed that cells at the margin of the infiltrated tissues emitted a strong yellow-orange fluorescence during exitation at 365 nm, 10 h.p.i. This fluorescence intensified at 24 hours and was observed especially within the main part of the infiltrated area that became necrotic (Fig. 10A). Observation at a higher magnification showed that the fluorescence was localized in walls and cytoplasm of cells adjacent to the non-infiltrated tissues of cotyledons. The intensity of the yellow fluorescence decreased at 72 hours. In contrast, neither sections from infected cotyledons of the susceptible line nor the uninoculated controls exhibited yellow fluorescence following treatment with Neu's reagents (Fig. 10B). These results strongly suggested that flavonoid compounds were induced following infiltration by *Xcm* in the resistant line, but not in the susceptible line (11).

Evan's blue staining showed that these flavonoids accumulated in the living cells found at the margin of the necrotic tissues. This indicates that these compounds are probaly first synthezised in response to *Xcm* in cells that are not yet infected. But phenol-like molecules were not cytolocalized after the use of a laccase-gold probe at the ultrastructural level, although this enzyme was successfuly used on other plant/pathogen systems (4). Flavonoids may be then translocated toward the HR cells that became dead, as reported for sesquiterpenoid phytoalexins in another cotton resistant line (16). Our data support the hypothesis that flavonoids produced early in infected cotton cotyledons play a role in the defense strategy of cotton.

2. Phytoalexin accumulation

Sesquiterpenoid aldehyde detection with SbCl3 is indicated by the red colour of tissues (10). After treatment of healthy cotyledon sections of both lines, only glands at the leaf surface were stained red. In infected resistant and susceptible cotyledons, no accumulation of terpenoids was observed during the first 48 h.p.i. (Table 1). A positive reaction to SbCl3 was seen from 2 days after inoculation in the necrotic area of the resistant line. In resistant lines, terpenoid phytoalexin accumulation was also demonstrated to occur during the first 72 hours by Essenberg and collaborators (19). These terpenoid phytoalexins likely act after flavonoids (Table 1), since the sesquiterpene cyclase activity, the first enzyme in the biosynthetic pathway of sesquiterpenoid defense compounds, increases only 20 h.p.i. (12).

3. Peroxidase

Microscopical detection of peroxidase activites

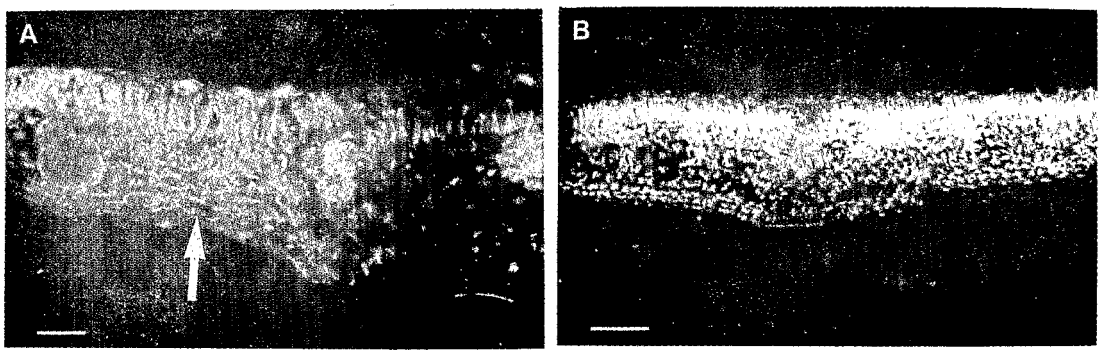


Figure 10 : Transverse sections of cotton cotyledons infiltrated with Xcm; sections were treated with the Neu's reagent for flavonoid detection and observed under UV illumination.

A. 24h after infection of the resistant cotton cultivar Reba B50, a strong yellow-orange fluorescence indicates that flavonoids are localized in the whole infiltrated area (arrow) both in the spongy and the palisade mesophylls. The tissues adjacent to the infected zone do not display any specific yellow fluorescence (Bar = 10 μm).

B. No flavonoid accumulation is observed on sections made in cotyledons of the infected susceptible cultivar as indicated by the absence of a yellow-orange fluorescence. The whitish fluorescence likely indicates the presence of other phenolic compounds (Bar = 10μm).

Table 1 : Autofluorescence, flavonoid and sesquiterpene accumulation in cotton cotyledons infected with Xanthomonas campestris pv malvacearum

Reagent	Specificity	Cultivar (a)	Reaction according to the time course of infection (hours) after infiltration (b)										Site of reaction (c)
			0	2	4	5	6	9	10	15	24	48	
Autofluorescence	Phenolics or	R	-	±	+	+	+	+	+	+	+	+	A
	terpenoid naphthols	S	-	-	±	±	±	±	±	±	±	+	A
	(yellow-green)												
Neu's	Flavonoids	R	-	-	-	-	-	+	+	+	+	+	B
	(yellow/orange) (d)	S	-	-	-	-	-	-	-	-	-	-	
Antimony chlorid	sesquiterpenoid	R	-	-	-		-	-	-	-	-	+	C
	aldehydes	S	-	-	-	-	-	-	-	-	-	-	
	(red)												

a : R = Reba B50, resistant; S = Acala 44, susceptible.

b : - = no response; ± = weak response; + = strong response.

c : A : yellow-green autofluorescence diffused in the infiltrated zone. At 4 hours, a strong fluorescence was seen at the margin of the infiltrated area in the resistant line.

B : yellow fluorescence was observed first in cells at the margin of the necrotic area, and later in cells within the necrosis of the resistant line.

C : terpenoids were detected in the necrotic area of infected cotyledons of the resistant line.

d : flavonoids appear yellow or yellow bright when illuminated with UV (365 nm) or blue light (420 nm), respectively.

(Table 2) in both resistant and susceptible cultivars were performed using guaiacol, tetramethylbenzidine, or diaminobenzidine. Peroxidase activities were detected as early as 2 hours in plant walls and middle lamella of cells in the HR lesions during the incompatible interaction (Figs. 7B, C). These activities were mainly located in the HR lesions. In parallel, we showed on isoelectric focusing

Table 2 : Histochemical and ultrastructural detection of peroxidase activity in cotton infiltrated with Xanthomonas campestris pv malvacearum

Cultivar	Time (a)	Reagent					
		guaiacol (b)		TMB (b)		DAB (c)	
		spongy	palissade	spongy	palissade	spongy	palissade
	0	-	-	-	-	-	-
	2	-	-	-	-	+ (f)	± (g)
	4	+ (d)	-	-	-	++	+ (g)
Resistant	5	+	+ (d)	-	-		
	6	+	+	+ (e)	-	+ (d)	+ (g)
	9	+	+	++	-		
	15	+	+	++	-		
	0	-	-	-	-	-	-
	2	-	-	-	-	±	-
	4	-	-	-	-	±	-
Susceptible	5	-	-	-	-		
	6	-	-	-	-	±	±
	9	-	-	-	-		
	15	-	-	-	-		

- = no staining; ± = weak staining; + = moderate stainig; ++ = strong staining.

a : hours after infection of cotyledons with the pathogen.

b : reagents used for the observation of peroxidase activity under the light microscope.

c : reagent used for ultrastructural localization of peroxidase activity.

d : peroxidase activity was detected in cell walls and the cytoplasm.

e : peroxidase activity was detected in cell walls and intercellular spaces.

f : peroxidase activity was detected in stomatal guard cells, in the cytoplasm and intercellular spaces of the spongy mesophyll cells.

g : peroxidase activity was detected in the cytoplasm.

gels that increase in activity of cationic peroxidase isozymes (pI 9-9.5) during the incompatible interaction was observed 3 h.p.i. only (Fig. 11A). Anionic peroxidase activities were also investigated according to the time course of HR development. Increase in the activity was spectrophotometrically evidenced 12 h.p.i. (32), corresponding to two acidic isozymes (Fig. 11B) located in cell walls and cytoplasm of the infected resistant tissues within the necrotic lesions (Table 2).

This body of arguments indicated that the cationic peroxidases are likely responsible for the generation of superoxide anions, in accordance with Bolwell and coworker's assumption (4) that AOS-generating peroxidases are wall-bound. In contrast, the function of the stimulated anionic peroxidases was not assessed. A recent study reported that plant peroxidases may also generate antimicrobial phenolics (28). Several phenols such as flavonols and flavanones have been reported to be converted into oxidized or polymerized compounds by activated vacuolar or cytoplasmic peroxidases (33, 34, 36). In this respect, ionically wall-bound peroxidases were suggested to be associated with the high level of flavonoids incorporated into the cell wall (38). In line with these observations, anionic peroxidases in infected cotton may be involved in flavonoid incorporation onto cell wall polysaccharides, as potent free radical scavenging enzymes.

IV Characterization of Systemic Acquired Resistance

When resistant cotyledons were previously inoculated with *Xcm* race 18, and leaves post-infiltrated 72 hours later with the same pathogen,

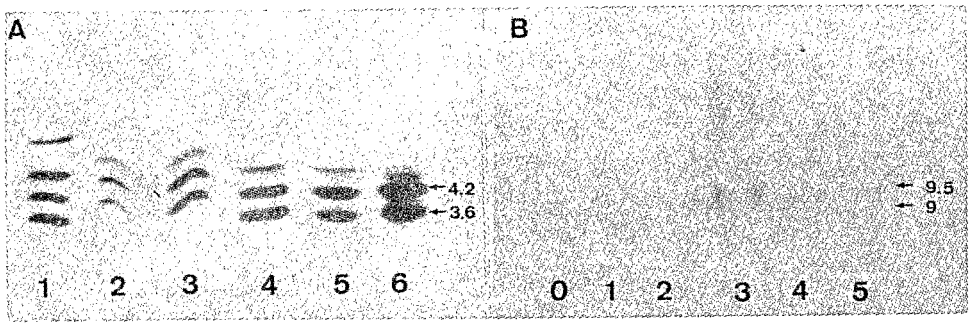


Figure 11: Isoelectrofocusing of peroxidase activities.

A. 10 hours following infection, the activity of two anionic isoforms (pI 3.6 and pI 4.2) evidenced by IEF after guaiacol staining increases in the infected resistant cotyledons (lane 6) as compared to water infiltrated resistant cotyledons (lane 5), infected (lane 4) and infected susceptible cotyledons (lane 3), and non treated cotyledons of both cultivars (lanes 1 and 2).

B. Analytical isoelectric focusing (IEF) according to time (0 to 5 hours; lane 0 to 5) of samples from resistant cultivar Reba B50 infected with Xcm race 18. Cationic peroxidase isozymes (pI 9 - 9.5) were separated on an IEF gel (pH 3 to 11) followed by guaiacol staining. Increase in the activity is observed 3h following inoculation (lane 3), but not at the other time following inoculation.

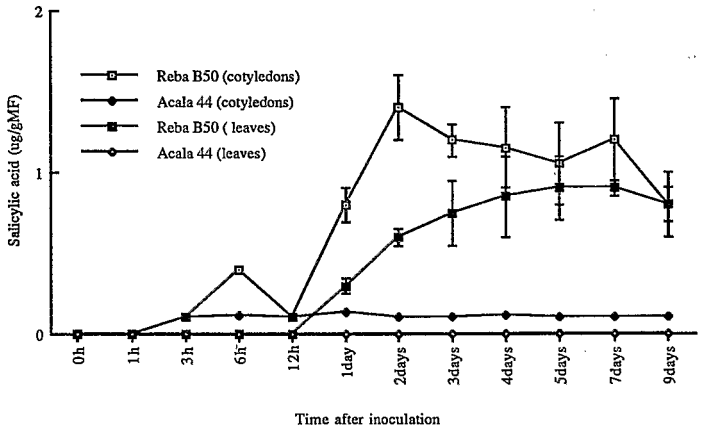


Figure 12 : Local and systemic detection of salicylic acid in Xcm-infected cotyledons of the resistant and the susceptible cultivars, and in the non-infected leaves.

In the infected cotyledons of the resistant plants (□), a peak of SA is detected 6 h following inoculation; a high level of SA is seen from 1 to 9 days after infection. In non-infected leaves (■), the level of SA increases from 12h to 9 days.
In the susceptible plants, no significant variation was seen in both the infected cotyledons (♦) and the non-infected leaves (◊)

more than 50% inhibition of the bacterial density in leaves was recorded. During the compatible interaction, 18% inhibition only was observed. Similarly, stimulation of anionic peroxidase activities following infection of resistant cotyledons with *Xcm* race 18, not only occurred in the necrotic lesions of cotyledons, but was also characterized in the whole infected plants 24 hours later. These observations revealed a possible systemic induction of peroxidase activities associated with a reduction in bacterial growth in late infected leaves.

Since salicylic acid (SA) has been widely associated with systemic acquired resistance (SAR) (35), the time course of SA accumulation in infected plants according to infection was investigated. Figure 12 shows two striking peaks 6 and 12 h.p.i. of the infected resistant cotyledons, while in leaves of the same plants SA was produced from 12 hours to 4 days. Infiltration of SA (2mM) into non-infected resistant cotyledons induced a significant increase in peroxidase activities between 3 and 6 hours following infiltration in cotyledons, and between 12 and 24 hours in leaves of the same plants. Isoeclectrofocusing revealed that two anionic peroxidase isoforms were responsible for these increasing activities.

Taking together, these data indicated that SA plays a key role in the induction of SAR in cotton infected with *Xanthomonas*. It has been demonstrated elsewhere that SA could be at the onset of SAR signalling (15, 35). Accordingly, recent experiments showed that in infected cotton plants that AS was not transported from cotyledon to leaves, suggesting that another signal was involved in activation of SAR, including the increase in leaf SA content.

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