anatomical and chemical characteristics that occur both constitutively and as a active defense mechanisms. A high degree of correlation in the expression of resistance of the plants has been dermonstrated for a wide range of genotypes. In *Gossypium* species, the resistance results from 22 so-called B-genes (6). The Reba B50 contains the B_2-B_3 gene combination which induces an hypersensitive reaction (HR) of the plants when infected with Xcm race 18, while the Acala 44 line, with no major genes for resistance, is susceptible to blight.

Work on cotton resistance to bacterial blight has been mainly focused on terpenoid molecules, the major antimicrobial metabolites produced by cotton. Some were idedentified from leaves and cotyledons of inoculated resistant plants, at a sufficient level to inhibit *in vivo* growth of the pathogen at infection sites (3,4,5). *De novo* synthesis of flavonoids was also histochemically evidenced close to the infection sites during the incompatible interaction (1). The associated ultrastructural observation of such phenol producing-cells indicated that these phenolics accumulated in callose-rich papillae and host cell walls.

The role of plant POx during interactions between plant and Xanthomonas is poorly documented, although the induced-HR by this pathogen was more widely studied (12). Venere et al. (13) showed that a POx activity increased within resistant cotton cotyledons syringe-injected with 10^8 viable cells of Xcm. More recently, an increased activity of a cationic POx was associated with an incompatible relation between rice and the vascular bacteria *X. oryzae pv. oryzae* (10). This POx, immunolocalized in xylem vessels was suspected to be involved in ligninsynthesis (14).

In the present study, we investigated POx isoenzymes involved in interactions between two cotton lines (Reba B5 and Acala 44) and two races of Xcm (race 18 and race :20). We evidenced that the activity of two anionic POx increased early during the incompatible interaction, while they did not, or weakly, in the infected susceptible plants. They were also shown to be first restricted to infection sites where the HR developed, and then systemically induced in the whole infected plants.

(f. w.)] was assayed in a 1 mL reaction medium (0.05 M / pH 6 sodium phosphate buffer containing 0.2% guaiacol and 0.03% H_2O_2). The activity, determined by measuring the slope from the linear increase in absorbance at 470 nm, was expressed as ΔO_{470}/min/mg tissues.

Isoelectric focusing (IEF). IEF was basically monitored according to Robertson et al. (11) on vertical gels, the pH gradient of which ranged from 2 to 10. After migration, POx isoforms were stained in a reaction medium containing 0.2% guaiacol, 0.03% 3-amino-9-ethylcarbazole and 0.03% H_2O_2 in 0.05M sodium phosphate buffer pH 6.

Histochemical localization of peroxidase activity. Peroxidase activity was detected in fresh cotyledon sections using 3,3',5,5'-tetramethylbenzidine (7) or guaiacol (8) as substrate. After incubation, the sections were rinsed in distilled water and observed with a light microscope. Control rnaterial was incubated in the medium (a) in the absence of H_2O_2 or (b) containing 0.05 M 3-amino-1,2,4-triazole (AT) in order to inhibit catalase if present.

activity in leaves and cotyledons and bacterial population during infection

SYMPTOMS	PEROXIDASE ACTIVITY (U/g)*		Cfu/cm2 cotyledon* X 10^6
	cotyledons	leaves	
HR t=8h	748 +/- 91	129 +/- 38	2.2 +/- 0.5
HR	1631 +/- 82	135 +/- 23	6.6 +/- 0.8
	4048 +/- 1012	354 +/- 12	70 +/- 10
	6890 +/- 370	500 +/- 67	150 +/- 12
	7120 +/- 204	700 +/- 64	200 +/- 14
	8004 +/- 144	1110 +/- 104	250 +/- 28
	9728 +/- 130	1360 +/- 137	239 +/- 31
	6100 +/- 132	1800 +/- 116	150 +/- 17
Dead of cotyledons	°	1778 +/- 104	°
°	735 +/- 128	129 +/- 31	2 +/- 0.5
°	1402 +/- 79.2	110 +/- 18	7 +/- 0.7
°	1300 +/- 175	119 +/- 47	90 +/- 9
°	2300 +/- 280	420 +/- 52	415 +/- 35
ater soaked lesions	2450 +/- 210	602 +/- 39	3000 +/- 25
"	2616 +/- 116	756 +/- 64	3700 +/- 24
"	2636 +/- 80	1056 +/- 104	3980 +/- 15
"	2024 +/- 76	804 +/- 50	3500 +/- 37
ead of cotyledons	°	708 +/- 51	°
°	453 +/- 110	44 +/- 5	2 +/- 0.06
°	132 +/- 32	29 +/- 13	8.2 +/- 0.5
°	1083 +/- 140	90 +/- 38	99 +/- 10
°	1114 +/- 72	91 +/- 23	510 +/- 35
ater soaked lesions	1076 +/- 65	101 +/- 24	3700 +/- 52
"	832 +/- 46	80 +/- 14	4830 +/- 32
"	804 +/- 28	51 +/- 10	5210 +/- 56
"	804 +/- 24	55 +/- 5	4890 +/- 28
ead of cotyledons	°	43 +/- 16	°

Characterization of peroxidase isoenzymes by IEF. Four major soluble acidic POx isoenzymes were evidenced after IEF on polyacrylamide gel. They all were present in both non-inoculated and inoculated plants of the resistant as well as the susceptible lines. Their pI values were 3.6 and 4.2, 4.7 and 5.6, respectively. A very weak band was also be observe located at the 5.2 pH zone of the gel. In the Xcm race 18 infected resistant plants, the activity of the 3.6 and 4.2 pI isoforms (and those only) increased considerably during the infection, accounting for the increase in total peroxidase activity shown in the corresponding cotyledon extract. In the infected susceptible plants no similar differential stimulation was observed. When leaf extracts were subject to IEF separation, comparable isoperoxidase patterns were observed, but specific increase in the activity of the 3.6 and 4.2 pI isoforms was not detected, even in leaves from resistant plants.

Histochemical detection of peroxidase activity. Using guaiacol as substrate, POx activity was detected in the spongy mesophyll cells of resistant cotyledons, at the infection sites, as early as 4h after infiltration of resistant cotyledons. In sections from infected cotyledons of the susceptible cv, no significant reaction was found. No staining was observed in sections when the incubation medium failed in hydrogen peroxide.

Cytochemical localization of peroxidase activity at the ultrastructural level. Electron microscopy on sections of infected resistant cotyledons revealed that activity was detectable 2h after infection mainly in spongy mesophyll cells, and weakly in palissade mesophyll cells. The middle lamella bordering intercellular spaces was also strongly electron-dense. In infected cotyledons of susceptible plants, a weak POx activity was observed in the tonoplast, but not in the middle lamella and the cell walls. No electron-dense areas were shown when sections were incubated in hydrogen peroxide-less medium.

DISCUSSION

In a previous work, Venere (13) has shown that POx activity increased in *Xanthomonas*-infected cotyledons of resistant plants. An associated decline in bacterial number recovered from the inoculated tissues several hours after inoculation was also evidenced. Similar results were recorded in our study of the incompatible host-pathogen system Reba B50/Xcm race 18. In the compatible interaction, POx activity enhancement did not occur, and bacterial population increased during the whole time course of infection. Comparison of POx isoforrn patterns monitored by IEF electrophoretic gels during time course experiments indicated that among the five acidic forms that were characterized within cotyledons of the susceptible and the resistant varietes, intensity of the activity of two major isozymes (pI 3.6 and 4.2) enhanced in the resistant line after infection. POx activity was shown by cytochemistry to be restricted to the HR areas in the early stages of the infection process. A systemic enhancement of POx activity was also biochemically evidenced in the infected plant. Such a local and systemic enhancement of POx activity has already been reported after treatment of plants with different pathogens or chemicals (2).

Of interest was (a) that infection of Reba B50 with Xm race 20 (that has overcome resistance of this line), induced into the cotyledons an intermediate enhancement of POx activity as compared both with compatible Acala 44/Xcm race 18 interaction and the incompatible Reba B50/Xcm race 18, but without specific stimulation of the 3.6 and 4.2 pI isoforms, and (b) that no specific isoperoxidase was induced when systemic POx enhancement occurred in leaves. These observations suggest that the „peroxidase reaction" may be under two kinds of control, one depending resulting from activation of the B_2-B_3 resistance gene(s)

of Reba B50 by the *avr* gene(s) of Xcm race 18 leading, among others, to early induction of two specific isoperoxidases, and another one resulting from signaling that triggers systemic and more general defense reactions.

Investigations are in progress, namely (a) to purify the 3.6 and 4.2 pI isoforms and produce specific antibodies in order to cytolocalize these isoenzymes *in situ* and to follow their production during the time course of infection, and (b) to verify if the systemic induction of the POx reaction is associated with a systemic acquired resistance (SAR).

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