Int. J. Indust. Entomol. Vol. 12, No. 1, 2006, pp. 21~28

International Journal of *Industrial Entomology*

Peroxidase Activity during Leaf Infection of Mulberry (*Morus alba* L.) with Brown Leaf Spot Fungus *Myrothecium roridum*

Soumen Chattopadhyay*, Natraj Krishnan[1] and Manas D. Maji

Mulberry Pathology Laboratory, Central Sericulture Research and Training Institute, Berhampore 742 101, India.
[1]Silkworm Pathology Laboratory, Central Sericulture Research and Training Institute, Berhampore 742 101, India.

(Received 14 December 2005; Accepted 22 February 2006)

Peroxidase activity was measured in brown leaf spot pathogen (*Myrothecium roridum*) inoculated potted mulberry (*Morus alba*) during pre-symptomatic to various symptom development stages and compared with corresponding healthy leaf tissues. The enzyme showed a pH optimum of 7.0 and the activity was linearly increased up to 15 min of incubation. The peroxidase had a broad substrate specificity and the rates of oxidation were in the rank of pyrogallol > guaiacol > ascorbate at pH 7.0. Catechol at 10 mM inhibited 89% of guaiacol-peroxidase and 76% pyrogallol-peroxidase activities, indicated higher non-specific peroxidation in pyrogallol dependent assay system in mulberry than guaiacol. The optimum requirement for the guaiacol dependent assay was 0.2 ml (≈40 – 60 μg equivalent of protein) of crude enzyme source. Excepting the 8th leaf from the apex, the peroxidase activity did not vary appreciably in different leaf positions. In pre-symptomatic phases, an initial (1 to 5 min) rise of peroxidase activity was noticed in inoculated leaves, and then maintained a plateau up to 300 min. In contrary, non-infected tissue showed a slightly increased trend of enzyme level up to 420 min. In infected tissue, a sharp transient increase (3.1 fold) of peroxidase activity appeared between 300 – 420 min post infections. Afterwards, significantly different but steady maintenance of enzyme levels were observed in two treatments. On the other hand, during symptom development, a sharp increase in peroxidase activity was noticed up to 4th grade of lesion appearance (25.1% to 50% of leaf area infection), and then declined slightly. However, in non-infected but same age healthy leaves, such huge fluctuations of enzyme level did not apparent. A high positive correlation (R^2 = 0.92) between peroxidase activity and leaf spot development grades was also marked. The result implies that pre-symptomatic burst (between 1 – 5 and 300 – 420 min) and subsequent increased trend of guaiacol peroxidase activity may require for the symptomatic manifestation of *Myrothecium* leaf spot in mulberry.

Key words : Guaiacol-peroxidase, Necrotic lesion, Pre-symptomatic disease progression, Symptom development grades

Introduction

Mulberry (*Morus* spp.) is the sole food plant of silkworm *Bombyx mori* and *ca.* 195,000 hectare of lands are under mulberry cultivation in India. *Myrothecium roridum* is the causal organism of brown or tar colour leaf spot (MLS) in mulberry and the disease reported globally from temperate to tropical sericulture areas (Govindiah *et al.*, 1989; Murakami *et al.*, 2002). The MLS affects mulberry in at least 2 – 3 silkworm-rearing seasons at the Eastern parts of India and responsible for 8 – 12% of foliage loss during their peak season of incidences (Qadri *et al.*, 1999). Experimental bioassay of silkworm with MLS infected leaves indicates, the disease is responsible for about 30% of cocoons yield reduction (Pratheesh Kumar *et al.*, 1999). The multiple seasonal occurrences and significant economic loss to the mulberry grower warrants an in-depth study of the early events of MLS, which is less precisely known at present.

Accumulating reports indicate that peroxidase (POX; EC 1.11.1.7) plays a pivotal role during fungal pathogen-

*To whom correspondence should be addressed.
Mulberry Pathology Laboratory, Central Sericulture Research and Training Institute, Berhampore 742 101, India.
Tel: +91-3482-253962 extension 239; Fax: +91-3482-253962;
E-mail: soumenchatto@rediffmail.com.

esis and increased activity of the enzyme during infection is mostly host originated (Edreva *et al.*, 1989). At the time of infection, the POX is considered as a putative source of active oxygen species generation (AOS; Baker and Orlandi, 1995). On the other hand, the POX acts as a – a) scavenger of generated H_2O_2 during host – pathogen interaction (Lamb and Dixon, 1997) and b) cell wall cross-linker, mediating peroxidation, that partakes lignification in the tissue surrounding the site of pathogen intrusion (Schafer, 1994). Such lignification has been suggested as a mechanism for disease resistance, particularly during fungal pathogen attack (Hammerschmid and Kuc, 1982; Edreva, 1989). However, little attention has been paid to such important enzyme during the MLS infection process in mulberry.

In this work, we optimized some prerequisites of POX assay in mulberry, compared the pre-symptomatic pattern of enzyme activities during pathogen challenged and unchallenged conditions, and finally correlated different MLS symptom assessment grades with guaiacol POX activity in potted plants.

Materials and Methods

Plant, pathogen, and inoculation

Mulberry (*Morus alba* cv. S_1) plants were grown in earthen pots (diameter 36 cm, depth 25 cm) containing 15 kg mixture of soil and farmyard manure (1:1) with recommended agronomic inputs (Dandin *et al.*, 2001). Plants were maintained under natural light/dark cycle and temperature (21 – 34°C) regime. The experiment was conducted during their peak season of incidence during June to September. The potted 60 days old plants (after basal pruning) were infected with aqueous suspension (10^6 conidia ml^{-1}; 15 ml $plant^{-1}$) of viable *M. roridum* conidia. The pathogen strain was isolated from the fields of Central Sericulture Research and Training Institute, Berhampore and maintained on potato-dextrose agar in dark at 4°C with a subculture passage of 20 days. The plants were covered with a transparent polythene bag for 48 hrs after application of inoculum.

Disease Assessment

We rated MLS symptom development on a 1 – 5 scale (Chattopadhyay *et al.*, 2002). The scale was as follows: 0 = no infection, 1 = 0.1% – 5% leaf area infected, 2 = 5.1% – 10% leaf areas infected, 3 = 10.1% – 25% leaf areas infected, 4 = 25.1% – 50% leaf areas infected, 5 = 50.1% and above area infected. The percent disease index (PDI disease severity) was calculated according to the following formula:

$$PDI = \frac{\sum \text{all numerical ratings } (1-5)}{\text{No of leaves scored X maximum grade } (5)}$$

Peroxidase assay

POX was assayed following the method of Tiedemann (1997) with slight modifications. For each disease grading scale, six leaf discs (6 × 10 mm diameter = *ca.* 90 – 100 mg fresh mass) were removed from both sides adjacent to the infected/inoculated areas or from the same areas of uninfected/non-inoculated leaves. The discs were ground with a chilled mortar and pestle in 2 ml of cold extraction mixture containing 40 mM sodium phosphate (Na_2HPO_4/ NaH_2PO_4) buffer (pH 7.0), 1% (w/w) polyvinyl polypyrolidone, 0.3 mM EDTA. Additionally, for ascorbate-POX, 5 mM ascorbic acid was added to the extraction medium. The homogenate was filtered through 4 layers of cheesecloth and centrifuged (Sorvall RC-5C) at 10,000 *g* for 15 min. The supernatant was used as a crude POX enzyme source.

The assay mixture contained 40 mM sodium phosphate buffer (pH 7.0), 0.1 mM Na-EDTA, either of 5 mM guaiacol, pyrogallol or ascorbate, 10 mM H_2O_2 and 0.2 ml of crude enzyme source. All assays were performed with a final reaction mixture of 1 ml. The incubation periods were 5 to 20 min. The change in absorbance was measured in Shimadzu model 160 A spectrophotometer. Absorbance was monitored at 290 nm for ascorbate oxidation (Nakano and Asada, 1981), 470 nm for guaiacol oxidation (Reuveni *et al.*, 1992) and 490 nm for pyrogallol oxidation (Kar and Mishra, 1976). To ascertain the specificity of POX substrates, assay was performed with 10 mM of catechol according to Snell and Snell (1971). Each assay was repeated twice with triplicate replications per occasions. One unit of POX activity was defined as the amount of enzyme giving, under assay conditions, a change in absorbance of 0.1 min^{-1}. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Statistical analysis

All data were subjected to an analysis of variance (ANOVA) using Microsoft Excel ver.-4. The LSD at 5% level for significant F-values and correlation coefficient were determined in appropriate cases.

Results and Discussion

Optimum conditions for peroxidase activity

Apparently, the crude enzyme source used for mulberry POX assay showed 28% more preference to pyrogallol

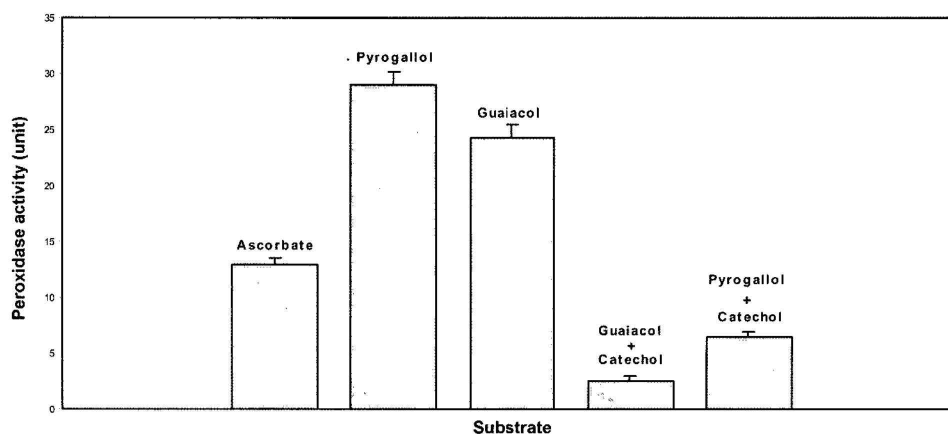

Fig. 1. Optimization of substrate specificity of peroxidase assay system in mulberry cv. S_1. Vertical bars are mean ± SE (n = 3). Incubation temperature was 25°C and time 15 min. Catechol (10 mM) was added to the assay mixture prior addition of specific substrate and enzyme source.

than guaiacol as substrate; while, the ascorbate was least preferred (Fig. 1). However, addition of 10 mM catechol in the assay mixture, an orthodiphenol inhibitor of POX, responsible for breakdown of enzymatically generated H_2O_2 (Snell and Snell, 1971; Nag *et al.,* 2000), reduced the guaiacol-POX and pyrogallol-POX activities 76% and 89% of their respective catechol untreated controls. It indicates a non-specific peroxidative oxidation was more in pyrogallol dependant assay in mulberry. Besides, in most of the pathogenesis induced POX assessment, guaiacol, rather pyrogallol was considered as an ideal substrate (Cvikrova *et al.,*1994; Polle *et al.,*1997; Luhova *et al.,* 2003). Therefore, in subsequent experiments we measured guaiacol dependent POX.

The optimization of pH of the assay buffer, substrate concentrations, quantities of crude enzyme sources and incubation time of the assay mixture were determined as these factors are reported to be varied in different plant systems (Bestwick *et al.,*1998) and less defined in mulberry. An incubation time of 15 min (Fig. 2) at pH 7.0 (Fig. 3) with 5 mM guaiacol (Fig. 4) and crude enzyme source of 0.2 ml (40 – 60 µg equivalent protein; Fig. 5) were optimum for mulberry leaf POX assay. The guaiacol-POX assay system is fairly stable at the temperature range of 23°C to 32°C (data not shown). Moreover, maximum POX activity was noticed at 8th leaf from the top of natural mulberry canopy at 40 days after pruning, irrespective of expression in unit protein or fresh weight functions (Fig. 6,7). Therefore, we maintained these assay conditions with 8th leaves of mulberry in subsequent assessments.

Peroxidase activity during pre-symptomatic development of MLS

Mulberry cv. S_1 is commercially cultivated in large scale

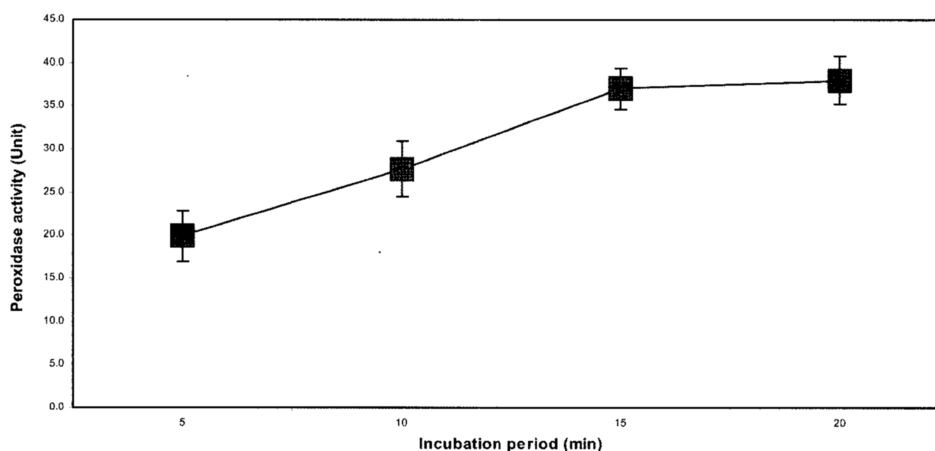

Fig. 2. Optimization of incubation period required for assay of guaiacol-peroxidase in mulberry. Vertical bars are mean ± SE (n = 3).

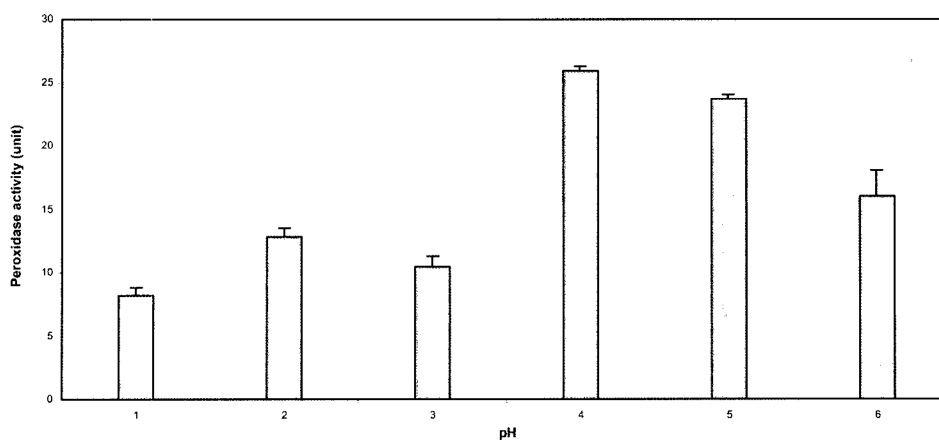

Fig. 3. Optimization of pH of the assay buffer required for assay of guaiacol-peroxidase in mulberry. Vertical bars are mean ± SE (n = 3).

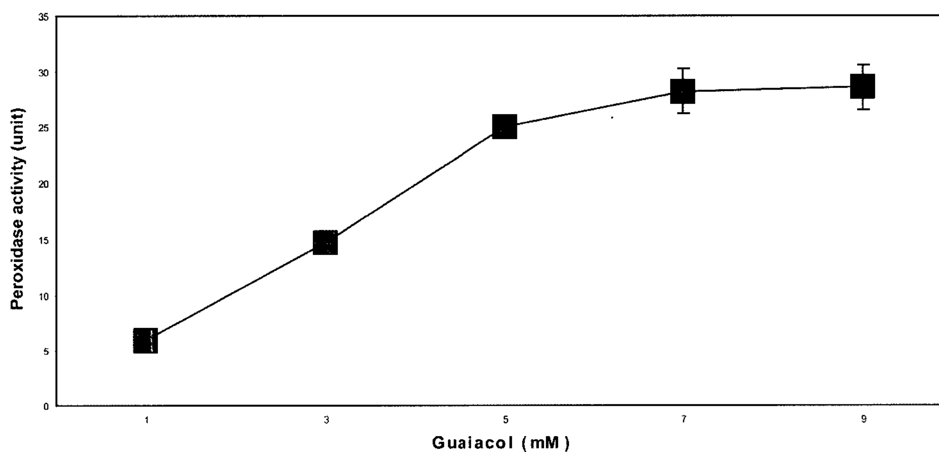

Fig. 4. Optimization substrate concentration required for assay of guaiacol-peroxidase in mulberry. Vertical bars are mean ± SE (n = 3).

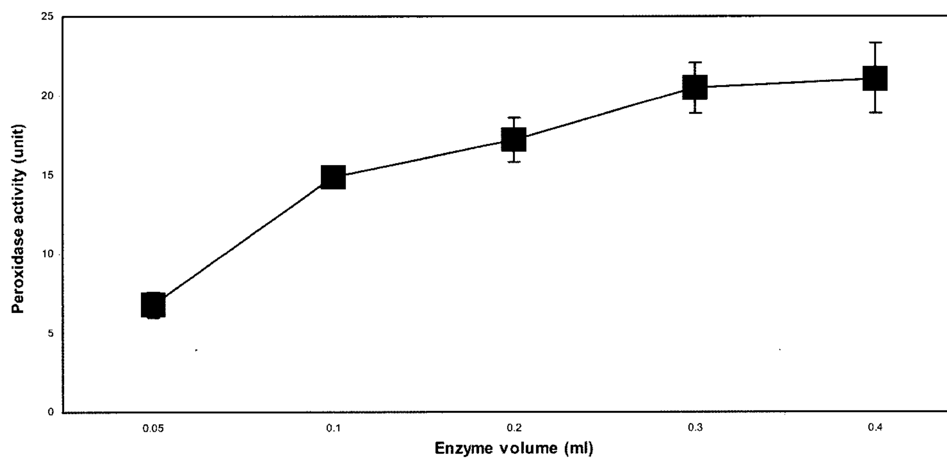

Fig. 5. Optimization of quantity of enzyme protein required for assay of guaiacol-peroxidase in mulberry. Vertical bars are mean ± SE (n = 3).

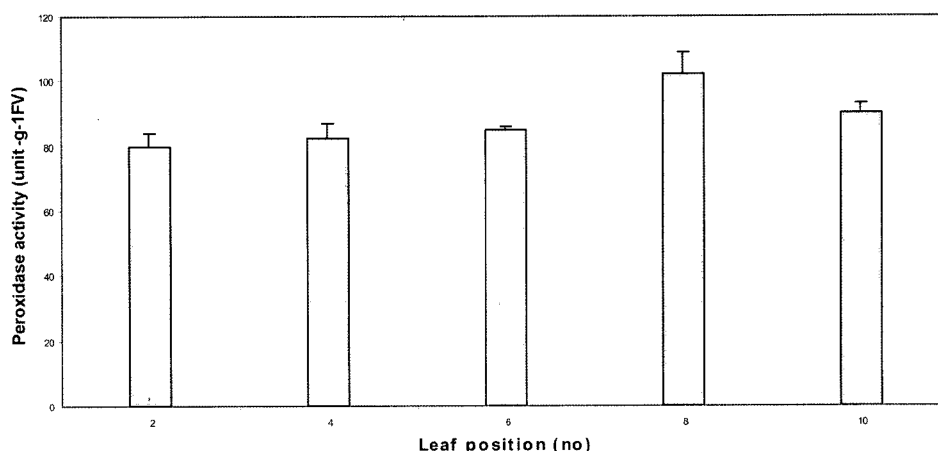

Fig. 6. Changes in peroxidase activity on the basis of fresh weight in different leaf positions of mulberry cv. S_1. Vertical bars are mean ± SE (n = 3).

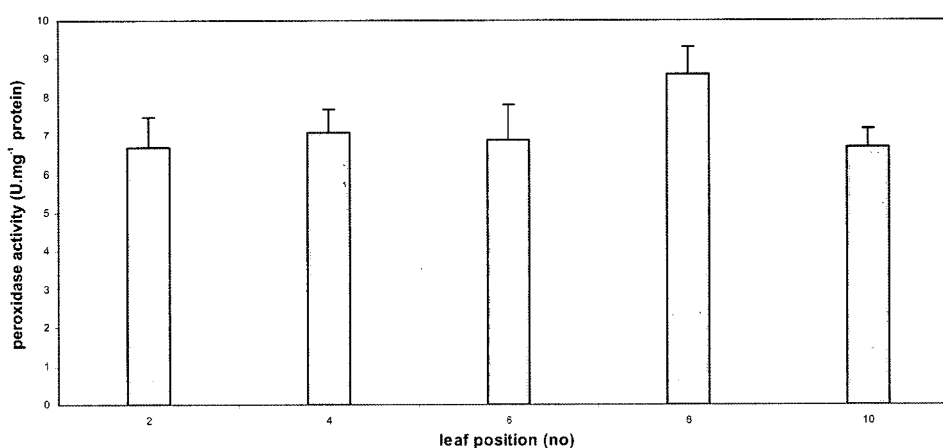

Fig. 7. Changes in peroxidase activity on the basis of protein content in different leaf positions of mulberry cv. S_1. Vertical bars are mean ± SE (n = 3).

at Eastern and North Eastern parts of India and the cultivar is moderately susceptible to MLS. The MLS disease on mulberry is characterized by necrotic spotting in the mature leaves during monsoon to autumn. Initial symptoms are small tar or brown spots that may develop into larger necrotic lesions of irregular shape with or without chlorotic halo. After spraying of inoculum onto potted 40 days old basal pruned plants, we measured the POX activities during early pre-symptomatic phases up to 4 days as well as with different symptom development phases up to 65 days. This period is ideal for silkworm consumption as well as MLS development (Pratheesh Kumar *et al.*,1999). The viable pathogen propagules inoculated tissues showed significantly higher level of POX activity than non-inoculated leaves when expressed either in unit fresh mass or protein basis (Fig. 8,9). The non-inoculated tissues maintained slightly increased trends of POX activity up to 420 min, thereafter showed an almost unaltered pattern. Contrarily, a complex biphasic rises and falls of POX activity during the same pre-symptomatic test regimen was noticed. Precisely, the enzyme level increased slightly (only 1.6 fold of the initial value) within 5 min of inoculation (Fig. 8), thereafter maintained a non-significant fairly steady plateau up to 300 min. Finally, a huge upsurge of the POX (3.1 fold of initial value) was detected at 420 min post inoculation, then again declined. Initial raise of POX activity was slightly more when the enzyme function expressed on unit of fresh mass than protein; while, the second peak was almost of similar amplitudes in both expression units. The ratio of POX activity in uninfected to infected leaves was 1.0 : 1.7 at the time of first peak (5 min) and 1.0 : 2.3 at second rise (420 min). Though biphasic responses of AOS generations during various pathogen attacks were previously reported in potato (Doke, 1983), tobacco (Keppler *et al.*, 1989) and soybean (Baker *et al.*, 1993), but such response is scant in case of POX

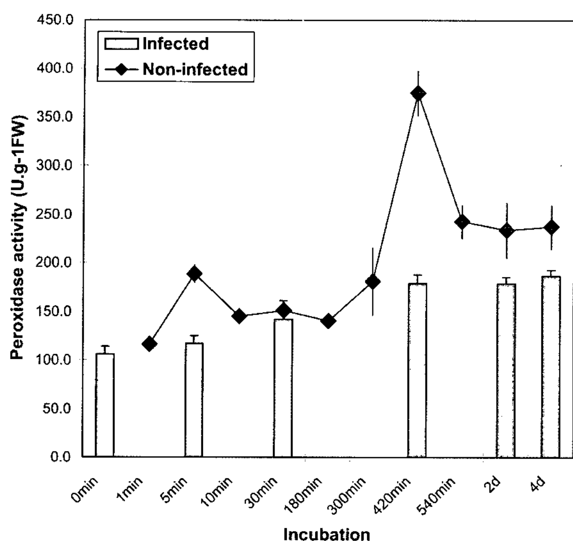

Fig. 8. Changes in peroxidase activity on the basis of fresh weight in *Myrothecium roridum* conidia inoculated (line) and non-inoculated (hatched bar) mulberry cv. S_1. Vertical bars are mean ± SE (n = 3).

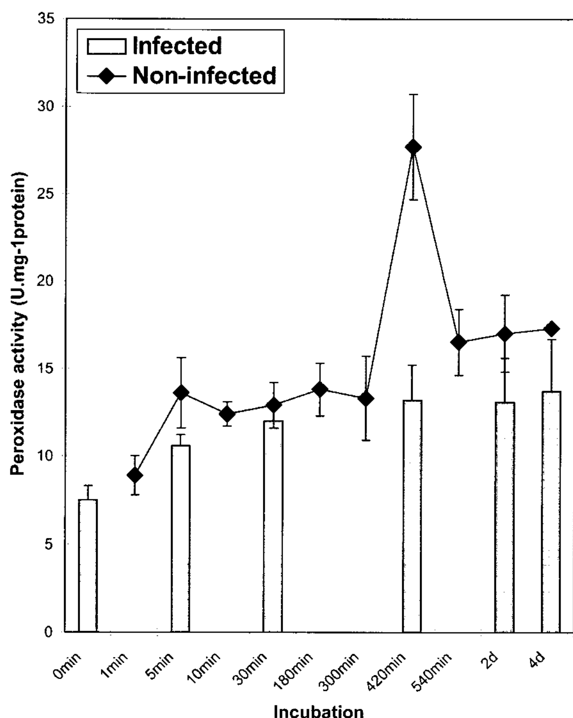

Fig. 9. Changes in peroxidase activity on the basis of protein content in *Myrothecium roridum* conidia inoculated (line) and non-inoculated (hatched bar) mulberry cv. S_1. Vertical bars are mean ± SE (n = 3).

activity. Plausibly, the AOS generation-a) initially hinders pathogen penetration by modulating cross-linking of cell wall at the site of contact, and b) subsequently generates

Table 1. Peroxidase activity in leaves of mulberry cultivar S1 during different stages of *Myrothecium* leaf spot symptom appearance in potted condition

Disease severity (grade)	Time after inoculation (d)	POX activity ($U \cdot g^{-1}$ fresh weight)	
		Inoculated	Non-inoculated
0	0	--	18.6 ± 0.6
1	4 – 7	23.3 ± 0.8	19.1 ± 0.3
2	8 – 11	31.4 ± 0.6	21.2 ± 0.4
3	12 – 15	38.6 ± 0.5	24.3 ± 0.4
4	16 – 19	43.1 ± 0.6	26.4 ± 0.5
5	20 – 24	42.2 ± 0.7	28.3 ± 0.4

Values are the mean ± SE (n = 3).

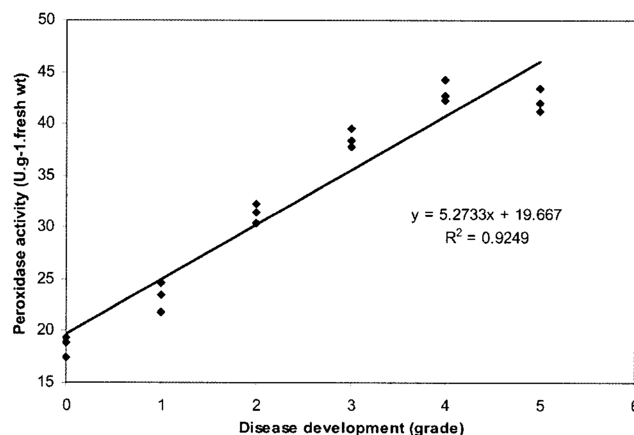

Fig. 10. Relationship of peroxidase activity with *Myrothecium* leaf spot symptom development in mulberry cv. S_1.

diffusible signal to distal plant parts (Alvarez and Lamb, 1997). Both the steps require an increased function of POX activity. Our work substantiates these observations, and perhaps first of its kind from a tree plant like mulberry.

Peroxidase activity and *Myrothecium* leaf spot severity

First visible symptoms of MLS appeared at 4 – 6 days of inoculation in potted plants and such lesions covered > 50% of leaf areas (5th grade) with in 20 – 25 days of infection at ideal condition. After infection, POX activity steadily increased with symptom development up to 4th grade in artificially inoculated plants thereafter declined. Overall elevation of enzyme activity was 2.3 fold from symptom grade 1 to 4 (Table 1) and maintained a positive correlation (R^2 = 0.92) with MLS symptom grades in inoculated tissues (Fig. 10). While, slightly increased but significantly lower amplitude (than inoculated tissue) of POX activity was noticed in non-inoculated leaves

throughout the test periods. The POX activity ratio was 1:1.7 in non-inoculated to inoculated tissues at 4th symptom development grade.

The chlorotic and necrotic symptoms on host tissue due to *Myrothecium* infection are seem to be initiated by a group of trichothecene toxins (Cutler and Jarvis, 1985). In mulberry specific strains of *M. roridum*, major virulent determining toxins are mainly roridine-E, myrotoxin-B (Murakami and Shirata, 1998). These toxin groups inhibited peptidyl transferase activity (Feinberg and McLaughlin, 1989) and impaired cell wall integrity (Kuti *et al.*, 1989) in hypersensitive tissues. The role of POX in the maintenance of cell wall integration during host pathogen interaction is established (Hamarschmidt and Kuc, 1995) and different isozymes of POX create an oxidative state in host cells and are associated with necrotic spot formation in different systems (Edreva, 1989; Graham and Graham, 1991; Reimers *et al.*, 1992). Though a corelational study relating the *M. roridum* induced leaf necrosis with AOS generation is yet to be established, but the present data showed that mulberry leaf tissue with MLS infection accumulated higher level of POX in general than the healthy tissue and POX activity was correlated positively with most of the stages of MLS development.

Most significantly, the results give a new line of evidence that pre-symptomatic biphasic courses of POX modulation are related to elevated level of POX activities during necrotic MLS symptom development. The absence of such modulation in *M. roridum* non-inoculated leaves further support the proposition.

Acknowledgements

Authors are gratefully acknowledging the Director, Central Sericultural & Training Institute for providing facilities.

References

Alvarez, M. E. and C. Lamb (1997) Oxidative burst mediated defense responses in plant disease resistance; in *Oxidative stress and the molecular biology of antioxidant defenses.* Sandalios, J. (ed.), Cold Spring Harbor Laboratory Press, New work.

Backer, C. J., N. M. Mock, J. A. Glazener and E. W. Orlandi (1993) Recognition responses in pathogen/nonhost and race /cultivar interactions involving soybean (*Glycene max*) and *Pseudomonas syringae* pathovars. *Physiol. Mol. Plant Pathol.* **43**, 81-94.

Baker, C. J. and E. W. Orlandi (1995) Active oxygen in plant pathogenesis. *Ann. Rev. Plant Pathol.* **33**, 299-321.

Bestwick, C. S., I. R. Brown and J. W. Mainsfield (1998) Localized changes in peroxidase activity accompany hydrogen peroxide generation during the development of a nonhost hypersensitive reaction in lettuce. *Plant Physiol.* **118**, 1067-1078.

Chattopadhyay, S., M. D. Maji, P. M. Pratheesh Kumar, K. K. Das and B. Saratchandra (2002) Response of mulberry brown leaf spot fungus *Myrothecium roridum* to different plant extracts. *Int. J. Indust. Entomol.* **5**, 183-188.

Cutler, H. G. and B. B. Jarvis (1985) Preliminary observations on the effects of macrocyclic tricothecenes on plant growth. *Environ. Expt. Bot.* **25**, 115-128.

Cvikrova, M., M. Hrubeova, M. Vagner, J. Machackova and J. Eder (1994) Phenolic acids and peroxidase activity in alfalfa *(Medicago sativa)* embryogenic cultures after ethephon treatment. *Physiol. Plant.* **91**, 226-233.

Dandin, S. B., J. Jaysawal and K. Giridhar (2001) Hand book of sericulture technologies. Central Silk Board, Bangalore, India.

Doke, N. (1983) Involvment of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. *Physiol. Plant Pathol.* **23**, 345-357.

Edreva, A. (1989) Host-parasite relations: Biochemistry; in *Blue mold of tobacco.* McKeen, W. E. (ed.), pp. 105-140, The American Phytopathological Society, St. Paul, MN.

Edreva, A., J. Georgieva and N. Cholakova (1989) Pathogenic and nonpathogenic stress affects on peroxidase in leaves of tobacco. *Environ. Expt. Bot.* **29**, 365-377.

Feinberg, B. and C. McLaughlin (1989) Biochemical mechanism of action of trichothecene mycotoxins; in *Trichothecene mycotoxicosis: pathophysiologic effects.* Beasley, V. R. (ed.), pp. 27-36, Boca Raton, CRC Press, FL.

Govindiah, K. Sengupta, D. D. Sharma, Gargi and V. Gunasekhar (1989) A new leaf spot disease of mulberry caused by *Myrothecium roridum* Tode Ex Fr. *Curr. Sci.* **58**, 398.

Graham, M. Y. and T. L. Graham (1991) Rapid accumulation of anionic peroxidases and phenolic polymers in soybean cotyledon tissues following treatment with *Phytophthora megasperma f.sp. glycinea* wall glucan. *Plant Physiol.* **97**, 1444-1455.

Hammerschmidt, R. and J. Kuc (1982) Lignification as a mechanism of induced systemic resistance in cucumber. *Physiol. Plant Pathol.* **20**, 61-71.

Hammerschmidt, R. and J. Kuc (1985) Induced resistance to disease in plants. Dodrecht, Kluwer.

Kar, M. and D. Mishra (1976) Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. *Plant Physiol.* **57**, 315-319.

Keppler, L. D., C. J. Baker and M. M. Atkinson (1989) Active oxygen production during bacteria induced hypersensitive reaction in tobacco suspension cells. *Phytopathol.* **79**, 974-

978.

Krishnan, N., S. Chattopadhyay, J. K. Kundu and A. Chaudhuri (2002) Superoxide dismutase activity in haemocytes and haemolymph of *Bombyx mori* following bacterial infection. *Curr. Sci.* **83**, 321-325.

Kuti, J. O., T. J. Ng and G. A. Bean (1989) Possible involvement of a pathogen produced trichothecene metabolite in *Myrothecium* leaf spot of muskmelon. *Physiol Mol. Plant Pathol.* **34**, 41-54.

Lamb, C. and R. A. Dixon (1997) The oxidative burst in plant disease resistance. *Annl. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 251-275.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Luhova, L., A. Lebeda, D. Hedererova and P. Pee (2003) Activities of amine oxidase, peroxidase and catalase in seedlings of *Pisum sativum* L. under different light conditions. *Plant Soil Environ.* **49**, 151-157.

Murakami, R. and A. Shirata (1998) The toxic components produced by *Myrothecium roridum,* causal fungus of leaf spot of mulberry, and its influence on the pathogenecity. *J. Seri. Sci.* Jpn **67**, 57-64.

Murakami, R., H. Kawakita and A. Shirata (2002) Infection behavior of conidia of *Myrothecium roridum* on mulberry leaf and cytological changes of leaf cells infected with the fungus. *Sericologia* **42**, 19-31.

Nag, S., K, Saha and M. A. Choudhuri (2000) A rapid and sensitive assay method for measuring amine oxidase based on hydrogen peroxide - titanium complex formation. *Plant Sci.* **57**, 157-163.

Nakano, Y. and K. Asada (1981) Hydrogen peroxide is scavenged by ascorbate - specific peroxidase in spinach chloroplast. *Plant Cell Physiol.* **22**, 867-880.

Polle, A., M. Eiblmeier, L. Shepparard and M. Murray (1997) Responses of antioxidative enzymes to elevated CO_2 in leaves of beech (*Fagus sylvatica* L.) seedlings grown under a range of nutrient regimens. *Plant Cell Environ.* **20**, 1317-1321.

Pratheesh Kumar, P. M., S. M. H. Qadri, S. C. Pal and B. Saratchandra (1999) Evaluation of few fungicides against two leaf spot diseases of mulberry (*Morus* spp.). *Bull. Sericul. Res.* **10**, 9-15.

Qadri, S. M. H., S. K. Gangwar, P. M. Pratheesh Kumar, C. Elangovan, N. K. Das, D. Maji and B. Saratchandra (1999) Assessment of cocoon crop loss due to leaf spot disease of mulberry. *Indian J. Seric.* **38**, 35-39.

Reimers, P. J., A. Guo and J. E. Leach (1992) Increased activity of a cationic peroxidase associated with an incompatible interaction between *Xanthomonas oryze pv. Oryze* and rice (*Oryza sativa*). *Plant Physiol.* **99**, 1044-1050.

Reuveni, R., M. Shimoni, Z. Karchi and J. Kuc (1992) Peroxidase activity as a biochemical marker for resistance of muskmelon (*Cucumis melo*) to *Pseudoperenospora cubensis. Phytopathol.* **82**, 749-753.

Schafer, W. (1994) Molecular mechanisms of fungal pathogenicity to plants. *Ann. Rev. Phytopathol.* **32**, 461-477.

Snell, F. D. and C. T. Snell (1971) Colorimetric method of analysis IV AAA; pp. 26–27, Van Nostrand Reinhold Co., New York.

Tiedemann, A. V. (1997) Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves with *Botrytis cinerea. Physiol. Mol. Plant Pathol.* **50**, 151-166.