Journal of Plant Biology, September 1999, 42(3) : 205-212

Foliar Responses of *Peristrophe bicalyculata* to Coal Smoke Pollution

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Study of the foliar responses of *Peristrophe bicalyculata* (Reth) Nees to the pollution caused by thermal power plant emissions revealed that the stomatal size, pore length, density, and index, as well as the photosynthetic rate and total chlorophyll content were reduced in *P. bicalyculata* plants at the polluted site in pre-flowering, flowering as well as post-flowering stage of plant growth. Contrary to this, stomatal conductance increased at each stage. The intercellular level of carbon dioxide was raised in the pre-flowering and flowering stages but decreased later at the polluted site.

Keywords: CO_2 uptake, *Peristrophe bicalyculata*, photosynthesis, pollution impact, stomatal responses

Environmental pollution and the energy crisis that mankind is facing are the outcome of urbanization and industrialization, as modern industrial development depends primarily on raw materials, energy, and transport. India has as many as 75 thermal power stations, releasing enormous amounts of sulphur dioxide, nitrogen oxides, carbon monoxide, hydrocarbon, fluorine, fly ash, and other waste products (Iqbal et al., 1999). Most of these pollutants are injurious to plants. By virtue of their unique position and specialized functions (gaseous exchange), leaves experience the maximum brunt of exposure to these pollutants, and accordingly have to undergo structural and functional alterations that often serve as markers of environmental pollution (Saxe, 1996).

The present report describes the effects of coal-smoke pollution on the form and function of leaves of *Peristrophe bicalyculata* plants growing exposed in the vicinity of the thermal power plant of Kasimpur in the Aligarh district of Uttar Pradesh, India.

MATERIALS AND METHODS

Plant Description

P. bicalyculata (Reth) Nees of the family Acanthaceae is an erect, hispid herb or under shrub 60-180 cm high, it is found in forest undergrowth, hedges, and wastelands almost throuthout India. Its leaves are ovate, acuminate, and pubescent; flowers are rose purple or pink in lax panicles; capsules are pointed and narrowed into a cylindrical stalk; and seeds are orbicular, papillose, and slightly rugose. The plant is used as fodder for horses and in southern India as a green manure. A yellowish brown essential oil (mp: 33-36°C) obtained by steam distillation of the plant shows tuberculostatic activity in vitro. It inhibits growth of various strains of *Mycobacterium tuberculosis* in concentrations of 15 to 20 µg/cc.

Site Description

The study was carried out around the Kasimpur Thermal Power Plant in the Aligarh district of Uttar Pradesh. The district lies in the north-west Uttar Pradesh (a northern state of India) in the fertile agricultural area of the Ganga-Jamuna doab, between 27° 29'N and 28° 11'N latitude and between 77° 29'E and 78° 38'E longitude. Kasimpur town is about 187 meters above the sea level.

This power plant, one of the three major thermal power plants of Uttar Pradesh, consists of three power stations with capacities of 90 MW, 210 MW, and 230 MW electricity generation, respectively. The complex runs on bituminous coal, which has 2.92% moisture, 22.20% ash, 31.68% volatile matters (including 0.49% sulphur), 5.61% hydrogen, 5.24% nitrogen, 20.23% oxygen, and 42.45% fixed carbon on the average. About 4194 metric tons coal/day is burnt, and the resultant emissions of SO_2, NO_x, and CO_2 from the stacks theoretically amount to 0.0169, 0.300, and 6.854 ppm/h, respectively (Table 1). The soil is composed of loam and a clayey type of loam at the different study sites, has a high pH and has a poor drainage system. The area experiences a dry and tropical monsoon type of climate.

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Table 1. Amount of gases released from the Thermal Power Plant Complex in different months. Measurements of gases in the stack were made at a height of 8 m.

Amount of SO_2 Months	Amount of NO_x		Amount of CO_2			pm/h
	kg/h	ppm/h	kg/h	ppm/h	kg/h	
Nov.	16437	0.016	294416	0.294	7784993	7.785
Dec.	18848	0.019	337696	0.338	11294414	11.294
Jan.	19695	0.020	352894	0.353	14647129	14.647
Feb.	18523	0.019	331869	0.332	14550399	14.550
March	20814	0.021	372920	0.373	17809853	17.810
April	18675	0.019	334590	0.335	1819826	1.820
May	17278	0.017	309442	0.309	02801652	2.802
June	14129	0.014	253152	0.253	2292021	2.300
July	11684	0.012	209354	0.209	1895479	1.900
Aug.	14503	0.015	259862	0.260	2352779	2.353
Sept.	15271	0.015	273623	0.274	2477369	2.477
Oct.	15533	0.016	278180	0.278	2519855	2.520
Average in winter (Nov.-March)	18863	0.019	337959	0.338	13217358	13.217
Average in Summer (April-June)	16694	0.017	299061	0.299	2304500	2.307
Average in Monsoon (July-Oct.)	14248	0.015	255255	0.255	2311371	2.312
Average	16783	0.017	300667	0.300	6853814	6.854

Methodology

Sampling was made from ten plants, at the pre-flowering, flowering, and post-flowering stages, growing at the polluted site (around the Kasimpur thermal power station) and on the reference site (Aligarh Muslim University Campus). Epidermal peels of fully expanded leaves were obtained using hot nitric acid (Ghouse and Yunus, 1972) and studied under a compound microscope. Fully developed stomata were considered for size measurement. The stomatal index was calculated by the formula of Salisbury (1927). Stomatal conductance, intercellular carbon dioxide concentration, and the photosynthetic rate were measured by a LI-6200 portable photosynthesis system (LI-COR; Lincoln, Nebraska, USA). Total chlorophyll content was estimated by the Arnon method (1949) using cold acetone, and the optical density was measured at 663, 645, 510 and 480 nm on a Beckman DU 640 spectrophotometer. Chlorophyll *a* and *b* and carotenoid contents were calculated by the formulas of MacLachlan and Zalik (1963) and Duxbury and Yentsch (1956). The data were analysed statistically to determine the significance level of the variations observed. Electron micrographs of the epidermal surfaces were obtained on an SEM (Philips 501 B).

RESULTS

Stomata

Length of the stomata in both upper and lower epidermises remained almost constant in all three stages of plant development in the control population. Compared to the control, it decreased significantly in both epidermises in the pre-flowering stage in plants growing under pollution stress. In the flowering stage, the decrease was greater in the upper epidermis than in the lower epidermis. The length considerably declined in both epidermal surfaces during the post-flowering phase. Per cent variations were maximum at the pre-flowering stage (in the lower epidermis) and flowering stage (in the upper epidermis). In both epidermises, stomatal width increased consistently with the age of the plant in the non-polluted area. Under the environmental stress it declined marginally in the upper epidermis at pre-flowering stage and in the lower epidermis at the flowering stage. The decrease was significant in other stages on both epidermal layers, being highly significant in the post-flowering stage (Table 2). The length of stomatal pore on both epidermises displayed an increasing trend with plant age at the reference site (Table 2). It decreased insignificantly on both surfaces in each

Table 2. Stomatal dimensions on the upper and lower foliar epidermises during different developmental stages of *P. bicalyculata* plants growing in the normal as well as polluted conditions. The values (Mean ± SD) represent a mean of 100 readings.

Parameters	Control	Polluted	% Variation
Length of stomata-UE (μm)			
Pre-flowering	31.50±4.63	24.75±2.25	21.42**
Flowering	32.67±4.00	25.02±2.22	23.41**
Post-flowering	32.85±4.51	26.68±3.28	18.78**
Length of stomata-LE (μm)			
Pre-flowering	24.75±2.25	21.80±3.00	11.91**
Flowering	24.75±1.25	24.30±1.22	1.81^{NS}
Post-flowering	26.55±3.25	24.90±2.78	6.21*
Width of stomata-UE (μm)			
Pre-flowering	17.10±2.15	16.33±2.17	4.50^{NS}
Flowering	19.48±3.91	17.90±2.09	8.11*
Post-flowering	22.63±4.90	18.45±2.42	18.47**
Width of stomata-LE (μm)			
Pre-flowering	16.47±2.41	15.00±2.21	8.92**
Flowering	17.23±2.07	16.33±3.17	5.22^{NS}
Post-flowering	21.73±3.30	18.27±2.34	15.92**
Stomatal pore length-UE (μm)			
Pre-flowering	19.60±2.17	19.17±2.30	2.19^{NS}
Flowering	20.07±3.28	19.53±2.79	2.69^{NS}
Post-flowering	21.15±2.69	20.07±2.24	5.10^{NS}
Stomatal pore length-LE (μm)			
Pre-flowering	17.20±2.86	16.65±2.88	3.19^{NS}
Flowering	17.82±2.70	16.78±2.34	5.83^{NS}
Post-flowering	19.17±2.30	18.45±2.16	3.75^{NS}

* = Significant at 5% level
** = Significant at 1% level
NS = Non-significant
UE = Upper Epidermis
LE = Lower Epidermis

stage of plant development at the polluted site. The maximum reduction occurred in the flowering stage (lower epidermis) and post-flowering stage (upper epidermis). The stomatal pores, wide open at the control site, tended to close under the stress (Fig. 1).

Table 3 provides data on stomatal density (SD) and stomatal index (SI). At the reference site, SD kept increasing with the age of the plant in both epidermises. It declined under pollution stress, non-significantly in the upper epidermis and significantly in the lower epidermis, during the pre-flowering phase. In the subsequent stages, a very significant decline was detected in both epidermal layers of the stressed population. Per cent variation was maximum at the flowering stage (in the lower epidermis) and post-flowering stage (in the upper epidermis). Stomatal

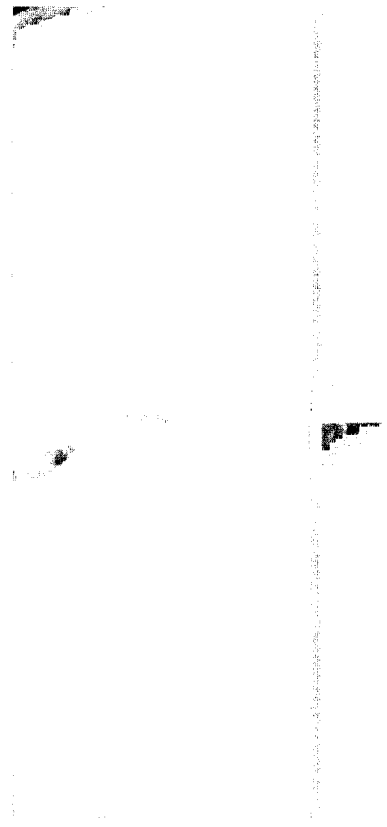

Figure 1. SEM pictures of the upper (A and B) and lower (C and D) epidermises of *P. bicalyculata* leaves. Stomata, wide open in the normal environment (A and C), tend to close down under the influence of coal-smoke pollution (B and D). All at 1800 X.

index (SI) also increased on both surfaces with growing plant age at the normal site. In the early stage of plant development, SI experienced slight reduction on the upper epidermis and a greater one on the lower epidermis of the stressed material. At later stages, it decreased markedly on both surfaces. Per cent variation was maximum at the flowering stage (in the upper epidermis) and post-flowering stage (in the lower epidermis) (Table 3).

Pigments

Quantitative estimation of chlorophylls *a* and *b* and carotenoids in leaves of both populations (Table 4) showed that pigment concentration decreased as the age of the plant at the normal habitat increased. The levels of chlorophylls *a* and *b* were significantly lower in the pre-flowering and flowering stages of the polluted population. However, at later stages, the loss was non-significant only. Carotenoid impairment due to coal-smoke pollution was insignificant in all stages

Table 3. Stomatal density and stomatal index on the upper and lower foliar epidermises during different developmental stages of *P. bicalyculata* plants growing in the normal as well as polluted conditions. The values represent a mean of 100 readings.

Parameter	Control	Polluted	% Variation
Stomatal density (UE)			
Pre-flowering	38.00±10.36	35.20± 7.92	7.36^{NS}
Flowering	63.60±10.88	44.00± 9.92	30.81**
Post-flowering	88.80±18.24	56.00±12.50	36.93**
Stomatal density (LE)			
Pre-flowering	44.00± 9.92	35.60± 5.76	19.09**
Flowering	77.60±12.92	46.00± 9.80	40.72**
Post-flowering	89.60±16.96	60.00±11.72	33.03**
Stomatal index (UE)			
Pre-flowering	62.80±11.80	60.92±11.52	2.99^{NS}
Flowering	106.60±12.02	72.12±14.12	32.34**
Post-flowering	166.08±13.36	125.04±14.16	24.71**
Stomatal index (LE)			
Pre-flowering	101.76±11.36	86.04±15.56	15.44**
Flowering	114.16±13.88	102.36±13.96	10.33*
Post-flowering	149.36±17.48	122.68±15.56	17.86**

* = Significant at 5% level
** = Significant at 1% level
NS = Non significant
UE = Upper Epidermis
LE = Lower Epidermis

Table 4. Pigment concentration in different developmental stages of *P. bicalyculata* plants growing in the normal as well as polluted conditions. The values represent a mean of 10 readings.

Parameter	Control	Polluted	% Variation
Chlorophyll *a* (µg/gm)			
Pre-flowering	0.90±0.04	0.66±0.03	26.66**
Flowering	0.68±0.07	0.56±0.05	17.64*
Post-flowering	0.60±0.06	0.54±0.03	10.00^{NS}
Chlorophyll *b* (µg/gm)			
Pre-flowering	0.70±0.04	0.36±0.09	48.50**
Flowering	0.57±0.05	0.32±0.06	43.85**
Post-flowering	0.33±0.08	0.25±0.05	24.24^{NS}
Carotenoids (µg/gm)			
Pre-flowering	0.34±0.05	0.24±0.09	29.41^{NS}
Flowering	0.29±0.05	0.22±0.09	24.13^{NS}
Post-flowering	0.26±0.04	0.20±0.06	23.07^{NS}

* = Significant at 5% level
** = Significant at 1% level
NS = Non-significant

of plant development. Per cent variation indicated a decreasing trend with growing plant age for all the three pigments (Table 4).

Table 5. CO_2 absorption and carbon assimilation in different developmental stages of *P. bicalyculata* plants growing in the normal as well as polluted conditions. The value represent a mean of 25 independent readings.

Parameter	Control	Polluted	% Variation
Stomatal conductance (m mole m^{-2} s^{-1})			
Pre-flowering	0.45±0.02	0.55±0.24	22.22^{NS}
Flowering	0.21±0.01	0.29±0.05	38.09*
Post-flowering	0.51±0.04	0.76±0.14	49.01*
Intercellular carbon dioxide (ppm)			
Pre-flowering	305.62±24.59	365.80± 7.81	19.69**
Flowering	219.86±15.45	275.59±34.72	25.34**
Post-flowering	180.60±14.01	267.05± 8.30	48.33**
Net photosynthesis (µ mole CO_2 m^{-2} s^{-1})			
Pre-flowering	7.53±3.09	2.65±0.57	64.80**
Flowering	11.81±1.42	7.50±0.95	36.49**
Post-flowering	24.52±4.59	20.45±3.68	16.59^{NS}

* = Significant at 5% level
** = Significant at 1% level
NS = Non-significant

Photosynthetic Capacity

Table 5 depicts the photosynthetic status of plants at the normal and the polluted sites. Stomatal conductance was the highest in the post-flowering stage and the lowest in the flowering stage at the control site. At the polluted site, it increased slightly in the pre-flowering stage and significantly in the subsequent stage. The maximum variation figured at the post-flowering stage. The intercellular carbon dioxide level decreased with growing plant age in normal conditions. At the polluted site, it significantly increased in the pre-flowering and flowering stages but markedly declined later, showing the maximum variation at the post-flowering stage. The net photosynthesis rate increased with plant age at the reference site. Under polluted conditions, it was relatively reduced, the reduction being significant in early two stages and only marginal during the post-flowering phase. Per cent variation showed a decreasing trend with growing plant age (Table 5).

DISCUSSION

The rate of absorption of air pollutants depends on their concentration gradient from the exterior to the interior of the leaf and on the stomatal dimensions, which play important role in determining the eventual extent of pollution impact on the plant body. In the present investigation, the length of stomata in *Peristrophe* leaves was significantly reduced in both

epidermises, and the maximum loss was encountered at the pre-flowering stage (lower epidermis) and flowering stage (under epidermis). The width of stomata was also reduced on both surfaces at the polluted site, more effectively during the post-flowering phase. However, the reduction in the stomatal pore length was insignificant throughout plant life on both epidermises. Evidence suggests that variations in pollution resistance capacity of different plant species are closely related to stomatal size. Jensen and Kozlowski (1975) have demonstrated that more SO_2 is absorbed by *Fraxinus americana* leaves, which have large stomatal apertures (high stomatal conductance), than by *Acer saccharum* leaves, which have small stomatal apertures (low stomatal conductance). Similar results with the leaf conductance values of ten species (trees and shrubs) in California have provided a good index of SO_2 uptake and resistance (Winner et al., 1982).

The effect of air pollutants often varies appreciably with differences in the stomatal diffusion resistance (SDR) which is a function of the stomatal index, stomatal size, and extent of the stomatal opening (stomatal aperture). Stomatal response to environmental changes helps in controlling the absorption of pollutants by plants, whereas the pollutants also influence the stomatal aperture. Effects of coal-smoke pollution (SO_2 being one of the major constituents) on the stomatal aperture are complex. The present investigation revealed that stomatal indices decreased significantly under the pollution load. This could be a natural mechanism adopted by the plant to cope with the atmospheric degradation. Likewise, the reduction in leaf size in the polluted atmosphere indicates retardation in growth as well as reduction in green surface area. Thus, the reduced foliar area in *P. bicalyculata* perhaps accommodates a lesser number of stomata thereby reducing the volume of abnoxious pollutants entering the leaves. Stomatal density (SD) was also markedly reduced at each state of plant development. The *Quercus rubur* produces leaves in warm summer temperatures that have a lower SD than their spring counterparts (Beerling and Chaloner, 1993). In poplar clones grown under an elevated level of CO_2, SD is reduced on both epidermises of expanding leaves on the upper portion of the plant, but mature leaves on the middle and lower plant parts remain unaffected (Ceulemans et al., 1995). The SD decreases in *Andropogon gerardii* and increases in *Salvia pitcheri* under an elevated level of CO_2 (Knapp et al., 1994). Ferris et al. (1996) have observed reduced SD on leaves exposed to a high temperature and CO_2 level.

In the present study, the stomatal index (SI) on both epidermal surfaces was low at the polluted site, marginally in early stage and significantly in the later stages of plant life. The maximum variation occurred in flowering stage (in the upper epidermis) and post-flowering stage (in the lower epidermis). In poplar clones, the SI was reduced under raised CO_2 levels in the expanding leaves of the upper plant parts (Ceulemans et al., 1995). It can thus be assumed that the stomata and epidermal cells respond to the pollution load differentially in the two epidermises at different stages of plant development. It is now known that SO_2 enters the leaves through stomata and reaches the intercellular spaces of mesophyll cells where it combines with water to form sulphurous acid, sulphate, and further to sulphite. There seems to be a close correlation between the size of stomatal aperture and the malate content of the leaves. The sulphite formed in leaves is thought to reduce the malate content (Kondo et al., 1984) and inhibit PEP carboxylase (Ziegler, 1973; Mukerji and Yang, 1974) and NADP malate dehydrogenase (Ziegler, 1974) involved in malate formation. Stomatal opening is also suppressed by oxaloacetate and bisulphite (Raghuvendra, 1980), suggesting thereby the regulation of stomatal opening by PEP carboxylase activity or malate content.

ABA induces closure of stomata or reduces the stomatal aperture, thus resisting entry of SO_2 and retarding the adverse effect. Decrease in stomatal aperture or stomatal closure may operate as an avoidance mechanism against inhibitory actions of a pollutant on photosynthesis (Kimmerere and Kozlowski, 1981). Thus the reduced size of stomatal aperture in the present study should be a mechanism for avoiding damage by SO_2 without major interferences with CO_2 supply.

Photosynthetic activity is based on the quality of chloroplast pigment as well as on the amount of photosynthetic area of the leaves. The total chlorophyll content in *P. bicalyculata* was relatively low under stress, the maximum loss being at the pre-flowering stage. Chlorophyll *b* was more significantly affected than chlorophyll *a*, as noticed earlier in various woody and non-woody plants (Joshi et al., 1993; Ajay and Subramanyam, 1996) possibly due to induced chlorophyllase activity or inhibition of chlorophyll *b* synthesis. However, both types of chlorophyll may be equally susceptible in some other species (Singh and Rao 1980; Singh et al., 1990a). Chloroplast damage by coal-dust pollution might cause reduction in chlorophyll concentration in the polluted leaves (Pandey

et al., 1991). Presence of Pb (Van Assche and Clijsters, 1990; Sinha et al., 1993) and SO_2 (Esmat, 1993; Ali, 1998) has also been suggested to inhibit chlorophyll biosynthesis. N deficiency also reduces chlorophyll content (Lawlor et al., 1989). Exposure to O_3 and SO_2 reduces chlorophylls and carotenoids in tomato (Khan and Khan, 1994).

Reduction of chlorophyll contents due to SO_2 pollution has been reported in many species (Ali, 1998). This could be due to the production of superoxide radicals as a result of reaction of sulphite with chlorophyll under illumination (Shimazaki et al., 1980; Williams and Banerjee, 1995). According to Singh et al. (1990b), the total chlorophyll and carotenoid content continues to decrease with increasing sodium metabisulphate ($Na_2S_2O_3$) concentration in tomato leaves. Chlorophyll *a* is thought to be degraded to phaeophytin under SO_2 effect by replacing Mg^{+2} ions from chlorophyll molecules. In chlorophyll *b*, SO_2 removes the phytol group of the chlorophyll *b* molecules (Rao and Le Blanc, 1966). It has been suggested that at pH 2.2-3.5, free H^+ ions are generated in the cell from splitting of HSO_3 into SO_3 and H^+, and displacing MG^{++} from the tetrapyrol ring of chlorophyll molecules to degrade them into pheophytin molecules (Shimazaki et al., 1980; Suwannapinunt and Kozlowski, 1980). This photosynthetically inactive brown pigment does not help in continuing the normal photosynthesis processes and causes the observed decrease in the total chlorophyll content in the polluted leaf samples.

Stomatal conductance in *Peristrophe* leaves increased slightly in the pre-flowering stage and markedly in the subsequent stages at the polluted site, as reported earlier for some trees and crop plants (Chappelka and Freer-Smith, 1995; Salam and Soja, 1995; Chandrashekar, 1997). On the contrary, stomatal conductance may decrease under the environmental stress (Field et al., 1995; Kull et al., 1996; Kellomaki and Wang, 1997), and this could be because of a low photosynthetic rate and a high intercellular CO_2 concentration (Farage et al., 1991). In the present study, the intercellular CO_2 level was raised due to pollution stress in the first two stages and then decreased in the post-flowering stage when the photosynthesis improved. Pandey and Pandey (1996) have observed a greater rate of carbon allocation in *Carissa carandas* due to photosynthetic acclimation of plants to the pollution load.

Photosynthesis is one of the foremost processes to be affected by coal smoke pollution. In the present investigation, the highest reduction (64.8%) in the rate of photosynthesis was recorded in the pre-flowering stage. This agrees with several earlier studies (Miller et al., 1991; Shan et al., 1996). The decline in photosynthesis due to pollutants may be via damage to the intersystem electron transport (Ishibashi et al., 1997), decrease in nitrogen content (Nakano et al., 1997), or decrease in PEP activity and concentration as a result of hydrolysis and mobilization from leaves (Joshi et al., 1993). Reduction in leaf areas could be an additional factor contributing to the decline. In *P. bicalyculata*, leaves are highly sensitive to the pollution load, and the extent of leaf damage is closely related to the stomatal response to pollution which in turn tells upon the overall physiology and growth of the plant.

ACKNOWLEDGEMENTS

The multifarious help provided by the fellow researchers, Rubina, Bhushra, Bhupinder and Gayoor Ali, during the course of this study is gratefully acknowledged.

Received February 22, 1999; accepted July 21, 1999.

LITERATURE CITED

Ajay, Subrahmanyam D (1996) Heavy metal induced changes in chlorophyll pattern in the tomato *Chlorella pyrenoidosa*. Ann Plant Physiol 10: 17-22

Ali ST (1998) Sulphur dioxide induced changes in the growth pattern of *Psoralea corylifolia* at different stages of development. Unpublished Ph.D. thesis. Jamia Hamdard, New Delhi

Arnon DI (1949) Copper enzymes in isolated chloroplasts of plants. Plant Physiol 4: 29-39

Beerling DJ, Chaloner WG (1993) The impact of atmospheric CO_2 and temperature change on stomatal density: Observations from *Quercus robur* lammas leaves. Ann Bot 71: 231-235

Ceulemans R, Vanpraet L, Jiang XN (1995) Effects of CO_2 enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus*). New Phytol 131: 99-107

Chandrashekar TR (1997) Stomatal responses of *Hevea* to atmospheric and soil moisture stress under dry subhumid climatic conditions. J Plantation Crops 25: 146-151

Chappelka AH, Freer-Smith PH (1995) Predisposition of trees by air pollutants to low temperatures and moisture stress. Environ Pollut 87: 105-117

Duxbury AC, Yentsch DS (1956) Plankton pigment nomography. J Air Pollut Contr Ass 16: 145-150

Esmat AA (1993) Damage to plants due to industrial pollution and their use as bioindicators in Egypt. Environ Pollut 81: 251-255

Farage PK, Long SP, Lechner EG, Baker NR (1991) The sequence of change within the photosynthetic apparatus of wheat follwing short term exposure to ozone. Plant Physiol 95: 529-535

Ferris R, Nijs I, Behaeghe T, Impens I (1996) Elevated CO_2 and temperature have different effects on leaf anatomy of perennial ryegrass in spring and summer. Ann Bot 78: 489-497

Field CB, Jackson RB, Mooney HA (1995) Stomatal responses to increased CO_2: Implications from the plant to the global scale. Plant Cell Environ 18: 1214-1225

Ghouse AKM, Yunus M (1972) Preparation of epidermal peels from leaves of gymnosperms by treatment with hot 60% HNO_3. Stain Technol 47: 322-324

Ishibashi M, Sonoike K, Watanabe A (1997) Photoinhibition of photosynthesis during the rain treatment: Intersystem electron transfer chain as the site of inhibition. Plant Cell Physiol 38: 96-146

Iqbal M, Srivastava PS, siddiqi TO (1999) Anthropogenic stresses in the environment and their consequences, *In* M Iqbal, PS Srivastava, TO siddiqi, eds, Environmental Hazards: Plants and People, CBS Publishers, New Delhi, pp 1-40

Jensen KF, Kozlowski TT (1975) Absorption and translocation of sulphur dioxide by seedlings of four forest tree species. J Environ Qual 4: 379-381

Joshi UN, Arora SK, Luthra YP (1993) SO_2 induced changes in CO_2 fixation and photosynthetic pigments in *Sorghum bicolor* leaves Ann Biol **9:** 102-108

Kellomaki S, Wang KY (1997) Effects of elevated O_3 and CO_2 on chlorophyll fluorescence and gas exchange in scots pine during the third growing season. Environ Pollut 97: 17-27

Khan MR, Khan MW (1994) Single and interactive effects of O_3 and SO_2 on tomato. Environ Exp Bot 34: 461-469

Kimmerere TW, Kozlowski TT (1981) Stomatal conductance and sulphur uptake of five clones of *Populus tremuloides* exposed to sulphur dioxide. Plant Physiol 67: 990-995

Knapp AK, Cocke M, Hamerlynck P, Owensby CE (1994) Effect of elevated CO_2 on stomatal density and distribution in a C_4 grass and a C_3 forb under field conditions. Ann Bot 74: 595-599

Kondo N, Maruta I, Sugahara K (1984) Effects of sulphite on stomatal aperture size in *Vicia* epidermal peels. Res Rep Nat Inst Environ Stud Jpn No 65: 9-18

Kull O, Sober A, Coleman MD, Diclson RE, Isebrands JG, Gagnon Z, Karnosky DF (1996) Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO_2. Can J For Res 26: 639-648

Lawlor DW, Konttusi M, Young AT (1989) Photosynthesis by flag leaves of wheat in relation to protein, ribulose bisphosphate carboxylase activity and nitrogen supply. J Exp Bot 40: 43-52

MacLachlan S, Zalik S (1963) Plastid structure, chlorophyll concentration and free amino acid composition of a chlorophyll mutant of barley. Can J Bot 41: 1053-1062

Miller JE, Pursley WA, Vozzo SF, Heagle AS (1991) Response of net carbon exchage rate of soyabean to ozone at different stages of growth and its relation to yeild. J Environ Qual 20: 571-575

Mukerji SK, Yang SF (1974) Phosphoenol pyruvate carboxylase from spinach leaf tissue. Inhibition by sulphite ion. Plant Physiol 53: 829-834

Nakano H, Makino A, Mae T (1997) The effect of elevated partial pressure of CO_2 on the relationship between photosynthetic capacity and N content in rice leaves. Plant Physiol 115: 191-198

Pandey DD, Sinha CS, Tiwari MG (1991) Impact of coal dust pollution on biomass chlorophyll and gram characteristics of rice. Biol J 3: 51-55

Pandey J, Pandey U (1996) Adaptational strategy of a tropical shrub, *Carissa carandas* L., to urban air pollution. Environ Monitor Assoc 43: 255-265

Raghuvendra AS (1980) Chloride and nitrate stimulate stomatal opening and decrease potassium uptake and malate production in epidermal tissue of *Commelina benghalensis*. Aust J Plant Physiol 7: 663-669

Rao DN, Le Blanc F (1966) Effect of SO_2 pollution on the lichen algae with special reference to chlorophyll. Bryologist 69: 69-75

Salam MA, Soja G (1995) Bush bean (*Phaseolus vulgaris* L.) leaf injury, photosynthesis and stomatal functions under elevated ozone levels. Water Air Soil Pollut 85: 1533-1538

Salisbury EJ (1927) On the causes and ecological significance of stomatal frequency with special reference to the woodland flora. Phil Trans R Soc 216: 1-65

Saxe H (1996) Physiological and biochemical tools in diagnosis of forest decline and air pollution injury to plants, *In* M Yunus, M Iqbal, eds, Plant Response to Air Pollution, John Wiley & Sons, Chichester, UK, pp 449-487

Shan Y, Feng Z, Izuta T, Aoki M, Totsuka T (1996) The individual and combined effects of ozone and simulated acid rain on growth, gas exchange rate and water-use efficiency of *Pinus armandi* French. Environ Pollut 91: 355-361

Shimazaki KI, Sakaki T, Kondo D, Sughara K (1980) Active O_2 participation in chlorophyll destruction and lipid peroxidation in SO_2-fumigated leaves of spinach. Plant Cell Physiol 21: 1193-1204

Singh N, Rao DN (1980) Studies of the effects of sulphur dioxide on alfalfa plants especially under conditions of natural precipitation. Indian J Air Pollut Contr 3: 1-8

Singh N, Singh SN, Srivastava K, Yunus M, Ahmad KJ, Sharma SC, Sharga AN (1990a) Relative sensitivity and tolerance of some gladiolus cultivars to sulphur dioxide. Ann Bot 65: 41-44

Singh SN, Yunus M, Singh N (1990b) Effect of Sodium metabisulphite on chlorophyll, protein and nitrate reductase activity of tomato leaves. Sci Total Environ

81: 269-274

Sinha SK, Srivastava HS, Tripathi RD (1993) Influence of some growth regulators and cations on the inhibition of chlorophyll biosynthesis by leaf in maize. Bull Environ Contam Toxicol 51: 241-246

Suwannapinunt W, Kozlowski TT (1980) Effects of SO_2 on transpiration, Chlorophyll content, growth and injury in young seedlings of woody angiosperms. Can J For Res 10: 78-81

Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 13: 195-206

Williams AJ, Banerjee SK (1995) Effect of thermal power plant emissions on the metabolic activities of *Mangifera indica* and *Shorea robusta*. Environ Ecol 13: 914-919

Winner WE, Koch GW, Mooney HA (1982) Ecology of SO_2 resistance. IV. Predicting metabolic responses of fumigated trees and shrubs. Oecologia 52: 16-21

Ziegler I (1973) Effect of sulphite on phosphoenol pyruvate carboxylase and malate formation in extracts of *Zea Mays*. Phytochemistry 12: 1027-1030

Ziegler I (1974) Malate dehydrogenase in *Zea mays*: Properties and inhibition of sulphite. Biochem Biophys Acta 364: 28-37