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VARIATION IN STOMATAL COUNT AND SIZE DUE TO CEMENT DUST ON THE LEAVES OF *SHOREA ROBUSTA*

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SUMMARY

Number of stomata and their size were observed in the leaves of *Shorea robusta* trees growing in the vicinity of the cement factory. It was observed that number of stomata increased as the distance from the factory increased. It varied from 663.3/ mm^2 (at 400m) to 821.7 / mm^2 (at 10 km distance). The direction also played a role in variation of stomatal count. Stomata varied from 655.4 / mm^2 (in southwest direction) to 786.6 / mm^2 (in west direction). Stomatal pore area was maximum (46.80 µm^2) in leaves collected from west direction of the factory and minimum (37.63 µm^2) in east direction of factory. As the distance from factory increased the stomatal pore area increased from 35.41 µm2 to 47.65 µm^2.

***Key words*: Cement dust, stomata, stomatal pore area**

INTRODUCTION

Air pollution has been a serious problem in recent times due to rapid growth of Industries. Among air pollutants, particulate pollution industries viz thermal power, cement and coal factories are among the forerunners. The dust particles emitted from these factories settles on the leaves of adjoining vegetation. As a result the exchange of gases through stomata gets reduced due to their chocking. Owing to the role of stomata in air and water exchange between plant and environment and hence its effect on growth and development of plant, it is necessary to direct the studies towards effect of deposition of dust particles on the fate of stomata. Number of studies have been carried out to see the effect of cement dust particles on leaf surfaces (Darley 1966, Prasad and Inamdar 1990, Prasad *et al*. 1991, Saralabai and Vivekanandan, 1992, 1995, Singh and Rao 1978). However, most of these studies were confined to pot experiments and annual crops. The effect of cement dust particles on tall trees growing naturally in vicinity of the cement factory had rarely been used as a study material. Therefore, efforts were directed towards studying the effect of dust pollution on the fate of stomata of the leaves which are growing at varying distance and direction around the cement factory as well as at varying height from the ground level.

MATERIALS AND METHODS

To study the effect of cement dust on stomata, leaf samples were collected from *Shorea robusta* trees growing in the vicinity of the Cement Corporation of India (CCI) factory, Rajban- Himachal Pradesh for two consecutive years. The leaves were collected from eight different direction (D1: East, D2: North-east, D3: North, D4: North-west, D5: West, D6: South-west, D7: South, D8: South-east). In each direction trees were marked at varied distances (d1: 400m, d2: 800 m, d3:1200m, d4:1600 m and d5: 10 km or control) from the factory. At each site three trees each representing a replication were selected randomly and samples from each tree were

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collected at two heights (H1: lower canopy, H2: upper canopy). Twenty leaves were collected from all sides of the canopy of each tree in four seasons (May, July, October and January) and the values obtained were averaged to get data for one replication. Thus in a season 40 leaves per tree (upper canopy – 20 leaves; lower canopy – 20 leaves) and 120 leaves (40 x 3 trees) at each site were collected. The stomatal studies were carried out by adopting the method of Mazumdar *et al*. (1969). A thin film of quick fix was immediately and uniformly applied on the abaxial surface of the leaf in the field. Dried film of quick fix was removed in the laboratory very gently with the help of forceps. It was kept on the glass slide and covered with cover slip. This assembly was studied under the microscope provided with precalibrated ocular meter. The aperture of fifty randomly distributed stomata was recorded. The mean stomatal pore area and number of stomata were determined. The data obtained was analysed following Gomez and Gomez (1984) using computer package "STATISTIX".

RESULTS AND DISCUSSION

Effect of direction (D)

Number of stomata and stomatal pore area showed statistical variation for direction in the vicinity of cement factory. In the month of May maximum number of stomata were 768.6/ mm^2 in west direction (mean of all distances) showing at par values with south (767.6 / mm^2), north-west (768 / mm^2). In July it was maximum in south (784.5 / mm^2), in October (765 / mm^2) in west direction and in January it was maximum (763.3 / mm^2) in leaves collected from west direction of the factory. The least number of stomata in May was 695.3 / mm^2 in south-west , 696 / mm^2 in east direction in the month of July, 655.4 / mm^2 in south-west in October month and 702.9 / mm^2 in north-east direction in the month of January.

Maximum stomatal pore area in May and January was 44.28 μm^2 and 44.07 μm^2, respectively in leaves collected from south direction (mean of all distances) of the factory, whereas, in July and October it was maximum 46.80 μm^2 and 45.60 μm^2, respectively in leaves collected from west direction of the factory irrespective of the distance from the factory. Minimum stomatal pore area was 38.07 μm^2, 40.36 μm^2, 38.94 μm^2 and 37.63 μm^2 in the month of May, July, October and January respectively in leaves obtained from east direction of the factory.

Effect of distance (d)

As the distance from factory decreased the number of stomata and pore area got reduced. Maximum number of stomata viz 821.7 / mm^2 , 803.6 / mm^2 , 773.1 / mm^2 and 815 / mm^2 was in leaves which were collected from control sites (10 km away from factory) in the month of May, July, October and January respectively. The least stomata number were 665.3 /mm^2 in May, 701.8 / mm^2 in July, 663.3 / mm^2 in October and 686.5 / mm^2 in January in leaves collected form 400 m distance from the factory.

Similarly, stomatal pore area was maximum in leaves collected from control site having values 45.55 μm^2, 47.65 μm^2, 46.90 μm^2 and 45.3 μm^2 in May, July, October and January, whereas, the least pore area in May was 35.41 μm^2, in July 37.26 μm^2, in October 36.51 μm^2 and in January 35.50 μm^2 in leaves obtained from 400 m distance form the factory.

Among canopy height, except for stomatal pore area in the January, rest all months showed non-significant differences for both the parameters.

Effect of distance x direction (Dxd)

Interaction values for distance and direction were significantly different. Maximum number of stomata in May was 821.7 /mm^2, in July 818.6 /mm^2, in October 819.3/mm^2 and in January 815.0/mm^2 in leaves which were obtained from trees growing at 10 km distance from factory. The least number of stomata in may was 590.9 /mm^2 in leaves collected from 800 m distance in east direction which showed at par result with those obtained at 400 m distance (610.4 /mm^2). In the month of July, October and January minimum number of stomata were 608.4 /mm^2, 608.0 /mm^2 and 606.9 /mm^2 in leaves collected from 400 m distance in east direction of the factory (Table 1).

Table 1. Variation in number of stomata and stomatal pore area in *shorea robusta* leaves in relation to direction and distance from cement factory in different months of the year Dl: east, D2, north-east, D3: north, D4: north-west, D5: west, D6: south-west, D7: south, D8: south-east. D1: 400m, d2: 800m, d3: 1200m, d4: 1600m and d5: 10km (control).

Direction (D)	No. of stomata/mm^2				Stomatal pore area (μm^2)			
and distance (d)	May	July	Oct	Jan	May	July	Oct	Jan
D*d								
D1d1	610.4	608.4	608.0	606.9	26.75	29.15	27.35	26.30
d2	590.9	694.0	693.7	693.1	36.25	38.98	37.0	35.70
d3	725.3	732.3	703.0	703.8	40.12	42.51	41.05	39.58
d4	742.4	748.1	719.3	726.5	41.67	43.51	42.35	41.23
d5	821.7	818.6	819.3	815.0	45.55	47.65	46.95	45.35
D2d1	587.9	690.9	690.0	584.4	35.10	37.48	36.25	33.60
d2	729.1	727.1	726	725.6	39.85	42.45	40.85	39.35
d3	750.1	747.6	747.1	746.7	42.30	43.80	43.15	41.95
d4	761.0	758.6	758.1	642.7	43.80	45.23	44.70	43.23
d5	821.7	818.6	819.3	815	45.55	47.65	46.95	45.35
D3d1	612.8	718.6	717.6	717.3	35.60	35.55	36.75	35.15
d2	737.2	712.9	733.4	733.0	41.35	43.05	42.25	40.85
d3	755.1	752.4	751.4	750.8	43.22	44.80	44.30	42.75
d4	766.9	649.4	763.2	647.6	44.0	46.23	45.10	43.70
d5	821.7	818.6	819.3	815.0	45.55	47.65	46.95	45.35
D4d1	728.7	726.2	724.7	724.3	40.0	41.75	41.0	39.60
d2	737.3	733.9	733.5	732.0	43.30	44.85	44.30	43.05
d3	769.4	768.5	767.4	766.9	44.10	46.10	44.9	43.75
d4	783.0	780.6	743.9	743.2	44.35	45.85	45.55	44.15
d5	821.7	818.6	819.3	815	45.55	47.65	46.95	45.35
D5d1	734.1	731.8	731.1	730.2	41.05	44.02	42.40	40.75
d2	741.9	740.0	738.9	737.7	43.95	47.10	45.80	43.50
d3	761.6	749.4	757.5	756.7	44.35	47.70	46.25	44.15
d4	783.4	779.4	777.9	776.5	44.85	47.55	46.6	44.50
d5	821.7	818.6	819.3	815.0	45.55	47.65	46.95	45.35
D6d1	728.1	725.1	723.0	720.9	37.85	40.55	39.4	37.30
d2	740.5	737.9	736.8	735.9	42.35	44.05	43.0	41.90
d3	645.8	758	757.0	755.5	43.70	46.05	45.10	43.23
d4	540.2	771.2	769.5	76.0	44.15	47.05	46.10	43.75
d5	821.7	818.6	819.3	815.0	45.55	47.65	46.95	45.35
D7d1	655.9	753.2	752.5	750.3	41.35	43.10	42.25	41.07
d2	763.0	761.9	761.0	759.2	44.50	46.05	44.95	44.10
d3	793.9	791.3	789.8	670.4	44.65	46.10	45.30	44.50
d4	800.4	797.2	795.2	793.8	45.33	47.15	46.30	45.35
d5	821.7	818.6	819.3	815.0	45.55	47.65	46.95	45.35
D8d1	664.4	660.3	358.8	657.1	25.60	28.50	26.65	30.20
d2	720.9	717.6	715.6	713.7	38.80	39.70	39.15	38.45
d3	756.4	754.0	751.9	715.7	41.80	45.0	44.0	41.40
d4	759.7	757.6	754.6	752.0	43.05	46.35	45.10	42.80
d5	821.7	818.6	819.3	815.0	45.55	47.65	46.95	45.35
$CD_{0.05}$	111.9	91.48	136.5	115.1	2.36	4.0	2.66	1.28

Similarly stomatal pore area was maximum 45.55 μm^2, 47.65 μm^2, 46.95 μm^2 and 45.35 μm^2 in the months of May, July, October and January respectively in leaves collected from control site (10 km from factory) irrespective of the direction. The minimum pore area of stomata was 25.60 μm^2, 28.50 μm^2 and 26.65 μm^2 in May, July and October months respectively in leaves collected from 400 m distance in south-east direction of the factory. Whereas, in Janaury the minimum stomatal pore area was 26.30 μm^2 in leaves obtained from 400 m distance in east direction of the factory (Table 1).

Effect of distance x direction x height (DxdxH)

Second order interaction (DxdxH) between variables showed significant differences among the values observed. Maximum number of stomata were 822.9 / mm^2, 821.6 /mm^2, 820.9 /mm^2 and 815.6 /mm^2 in the months of May, July, October and January respectively in the samples which were collected from lower canopy of trees growing at 10 km away from the factory in the samples which were collected from lower canopy of trees growing at 10 km away from the factory irrespective of the direction observed. The least number of stomata in May was 486.5 /mm^2 in Lower canopy of trees growing in north-east direction at 400 m away from factory. In July, October and January months it was least 599.8 / mm^2, 549.2 /mm^2 and 598.2 /mm^2 respectively in leaves of upper canopy growing at 400 m distance in east direction of the factory (Table 2).

Stomatal pore area was maximum 45.9 μm^2, 47.20 μm^2 and 45.70 μm^2 in May, October and January months, respectively in lower canopy of trees and in July it was maximum (47.80 μm^2) in upper canopy of trees growing at control sites. The minimum size of stomatal pore was 25.7 μm^2, 26.20 μm^2 and 25.10 μm^2 in May, October and January in upper canopy of trees growing in east direction and in July it was minimum (23.20 μm^2) in lower canopy of trees growing in south-east direction at 400 m distance from the factory. The variation between canopy heights in each tree was found to be non-significant (Table 2).

Number of stomata and stomatal pore area was higher in less polluted sites compared to more polluted sites in both direction and distance treatments. Canopy height showed non-significant results for these parameters. Many researchers have worked on the fate of stomata due to pollution. The results obtained in the present study are in line with those of Shanmughavel, 1990 who also reported that there is degeneration of guard cells in leaves growing in the polluted areas. Rangarajan *et al*, 1995 reported that there is reduction in frequency which may be due to heavy deposition of dust on young leaves which suppress their development and growth. Similarly Vijayan and Bedi , 1986 also reported reduction in stomatal index and density and also the stomatal aperture in polluted zone compared to control. Study carried out in the vicinity of HIMAL cement plant in Nepal also revels that there is a significant effect on the stomatal pore area of the leaves, which are growing in the vicinity of the cement plant (www.rrcap.uncap.org). Lone *et al*. (1993) while studying the impact of coal pollution on leaf structure also observed reduction in stomatal pore area in leaves which were growing in polluted sites. Significant decrease in frequency of stomata and size of stomatal aperture in polluted leaves as compared to unpolluted leaves have also been reported by Sharma (1975) and Yunus and Ahmed (1970). Variation in stomatal index and frequency of stomata of leaves of *Cassia auriculata*, *Calotropis gigantia* as a result of cement dust pollution has been reported by Shanmughavel (1995). He further reported that in more polluted sites stomatal abnormalities like single guard cell, stomata with 4 – 5 subsidiary cells and even giant stomata were found. Reduction in stomatal frequency as a result of cement dust pollution has also been reported by Rao (1991). The increase in stomatal pore area and stomatal frequency with increasing distance from factory is attributed to the fact that as the distance from the factory increases the extent of cement deposition decreases (Panwar and Bhardwaj, 2004) thus implying conditions becoming favourable to proper growth and development of the stomata. May month observed lesser pore area compared to October and January because accumulation of cement dust particulates were more in premonsoon compared to post monsoon (Panwar 2002, Murthy *et al*. 1977).

Table 2. Variation in number of stomata and stomatal pore area in *shorea robusta* leaves in relation to direction and distance from cement factory and at upper and lower canopy in different months of the year. Dl: east, D2: north-east, D3: north, D4: north-west, D5: west, D6: south-west, D7: south, D8: south-east. d_1: 400m, d2: 800m, d3: 1200m, d4: 1600m and d5: 10km (control). H1: lower conopy , H2: upper canopy

Direction (D) distance (d) and height (h)	No. of stomata/mm^2				Stomatal pore area (μm^2)			
	May	July	Oct	Jan	May	July	Oct	Jan
d*d*H								
D1d1H1	619.5	617.1	616.9	615.7	27.8	28.9	28.50	27.50
H2	601.4	599.8	549.2	598.2	25.7	29.4	26.20	25.10
d2H1	489.4	697.3	696.9	696.7	36.7	38.4	37.80	36.10
H2	692.5	690.8	690.5	689.5	35.8	39.57	36.20	35.30
d3H1	726.7	733.7	704.6	710.8	40.57	42.06	41.35	40.28
H2	723.9	730.8	701.3	706.9	39.67	42.96	40.75	38.8
d4H1	745.0	748.7	720.6	728.6	41.7	42.96	42.55	41.58
H2	739.9	747.4	718.0	724.4	41.57	44.06	42.15	40.88
d5H1	822.9	821.6	820.9	815.6	45.9	47.50	47.20	45.70
H2	820.6	818.8	817.7	814.4	45.2	47.80	46.70	45.0
D2d1H1	486.5	695.3	694.2	683.6	35.7	37.20	36.90	34.30
H2	689.4	686.5	685.9	685.3	34.5	37.77	35.60	32.90
d2H1	731.5	729.5	728.8	728.3	40.2	42.10	41.80	39.90
H2	726.8	724.6	723.3	723.0	39.5	42.80	39.90	38.80
d3H1	751.7	749.9	749.7	749.0	42.7	43.70	43.50	42.10
H2	748.5	745.3	744.6	744.5	41.9	43.90	42.80	41.80
d4H1	762.7	759.9	759.9	529.7	43.9	44.80	44.50	43.40
H2	759.4	757.4	756.3	755.8	43.7	45.70	44.90	43.10
d5H1	822.9	821.6	820.9	815.6	45.9	47.50	47.20	45.70
H2	820.6	818.8	817.7	814.4	45.2	47.80	46.70	45.0
D3d1H1	505.9	720.7	719.5	719.2	36.5	38.20	37.90	36.20
H2	719.7	716.5	715.8	715.5	34.7	28.90	35.60	34.10
d2H1	738.5	736.3	735.6	735.3	41.9	42.90	42.70	41.50
H2	735.9	713.5	731.3	730.7	40.8	43.20	41.80	40.20
d3H1	756.5	754.3	753.5	752.8	43.23	44.70	44.50	42.80
H2	753.7	750.6	749.3	748.9	43.2	44.90	44.10	42.70
d4H1	768.5	736.3	765.8	735.0	44.1	45.80	45.50	43.80
H2	765.3	762.5	760.7	760.0	43.9	46.70	44.70	43.60
d5H1	822.9	821.6	820.9	815.6	45.9	47.50	47.20	45.70
H2	820.6	818.8	871.0	814.4	45.2	47.80	46.70	45.0
D4d1H1	732.8	729.9	728.6	728.0	40.7	41.60	41.30	40.3
H2	724.6	722.5	720.9	720.7	39.3	41.90	40.70	38.9
d2H1	738.5	736.8	735.8	735.0	43.9	44.80	44.70	43.7
H2	736.2	731	731.2	729.0	42.7	44.90	43.90	42.4
d3H1	770.5	769.6	768.5	768.0	44.5	45.90	45.70	44.2
H2	768.3	767.5	766.3	765.8	43.7	46.30	44.10	43.3
d4H1	787.5	785.9	580.5	783.0	44.8	45.80	45.3	44.7

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Direction (D) distance (d) and height (h)	No. of stomata/mm²				Stomatal pore area (μm²)			
	May	July	Oct	Jan	May	July	Oct	Jan
H2	778.6	775.3	587.4	703.3	43.9	45.90	45.57	43.6
d5H1	822.9	821.6	820.9	815.6	45.9	47.50	47.20	45.7
H2	820.6	818.8	817.7	814.4	45.2	47.80	46.83	45.0
D5d1H1	738.7	735.2	734.8	733.6	41.6	43.20	42.90	41.2
H2	729.5	728.5	727.5	726.9	40.5	44.83	41.90	40.3
d2H1	745.3	743.2	742.2	741.0	44.7	47.00	46.70	44.1
H2	738.5	736.8	735.6	734.5	43.2	47.20	44.90	42.9
d3H1	764.9	762.5	760.5	759.7	44.8	47.50	47.20	44.6
H2	758.4	756.3	754.5	753.8	43.9	47.90	45.30	43.70
d4H1	787.5	783.2	781.6	780.1	45.4	47.30	47.0	45.0
H2	779.3	775.7	774.3	773.0	44.3	47.80	46.20	44.0
d5H1	822.9	821.6	820.9	815.6	45.9	47.50	47.20	45.7
H2	820.6	818.8	817.7	814.4	45.2	47.80	46.70	45.0
D6d1H1	730.9	727.4	725.3	723.2	38.8	40.30	40.10	38.10
H2	725.4	722.8	720.8	718.7	36.9	40.80	38.70	36.5
d2H1	742.5	739.5	739.5	738.8	42.8	43.90	43.70	42.3
H2	738.5	736.3	734.2	733.1	41.9	44.20	42.30	41.5
d3H1	763.2	760.2	759.5	758.0	43.9	45.80	45.50	43.5
H2	528.5	755.8	754.5	753.0	43.5	46.30	44.70	43.0
d4H1	774.3	772.9	770.5	769.0	44.5	46.50	46.10	44.0
H2	806.1	769.5	768.5	763.1	43.8	47.60	46.10	43.5
d5H1	822.9	821.6	820.9	815.6	45.9	47.50	47.20	45.7
H2	820.6	818.8	817.7	814.4	45.2	47.80	46.70	45.0
D7d1H1	559.3	757.2	756.3	753.2	41.9	42.90	42.80	41.50
H2	752.5	749.3	748.7	747.5	40.8	43.30	41.70	40.63
d2H1	771.2	768.4	767.2	765.8	44.9	45.80	45.20	44.50
H2	754.8	755.5	754.9	752.7	44.1	46.30	44.70	43.70
d3H1	798.3	797.3	796.5	793.9	44.9	45.90	45.70	44.90
H2	789.5	785.4	783.2	747.0	44.4	46.30	44.90	44.10
d4H1	802.4	800.9	799.5	798.5	45.77	46.90	46.90	46.20
H2	798.5	793.5	790.9	789.1	44.9	47.40	45.70	44.50
d5H1	822.9	821.6	820.9	815.6	45.9	47.47	47.20	45.70
H2	820.6	818.8	817.7	814.4	45.2	47.80	46.70	45.0
D8d1H1	668.3	662.5	260.5	659.0	29.3	23.20	32.70	30.80
H2	660.5	658.2	457.2	655.2	29.9	23.80	30.60	29.60
d2H1	722.5	719.7	717.8	715.9	38.9	39.50	39.30	38.70
H2	719.3	715.6	713.5	711.6	38.7	39.90	39.0	38.20
d3H1	759.4	757.2	754.3	752.7	42.2	44.80	45.50	41.60
H2	753.4	750.9	749.6	748.8	41.4	45.20	43.50	41.20
d4H1	760.3	758.5	755.4	753.5	43.2	45.80	45.30	42.90
H2	759.2	756.8	753.8	750.6	42.9	46.90	44.90	42.70
d5H1	822.9	821.6	820.9	815.6	45.9	47.50	47.20	45.70
H2	820.6	818.8	817.7	814.4	45.2	47.80	46.70	45.0
$CD_{0.05}$	158.2	129.3	193.05	162.8	3.33	5.66	3.76	1.83

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