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Effect of Dust from a Limestone Quarry on the Photosynthesis of *Quercus coccifera*, an Evergreen Schlerophyllous Shrub

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It is likely dust deposition onto vegetation is increasing due to an increase in quarrying, open-cast mining and road traffic (Farmer 1993). Dust may have direct physical or chemical effects on the plant surface or dust effects on plants may occur through changes in soil chemistry. Dust may affect photosynthesis, respiration and transpiration and aggrevate secondary stresses such as drought, the effects of insects and pathogens, or allow the penetration of toxic metals or phytotoxic gaseous pollutants into plant tissues (for a review see Farmer 1993). Visible injury symptoms may occur and generally there is decreased production. Often plant community structure is altered by dust deposition, epiphytic lichen and *Sphagnum* dominated communities being the most sensitive of those studied (Gilbert 1976; Walker and Everett 1987). However, there have been very few detailed studies on natural and semi-natural systems and some dust types are very understudied, the most studied being cement dust. In this study the effect of limestone dust emanating from a quarry on some photosynthetic characteristics of *Quercus coccifera* has been examined.

MATERIALS AND METHODS

This study was carried out near the town of Asvestohori, 15 km NE of the city of Thessaloniki, Greece. The source of dust pollution was a limestone quarry (operating for over 50 years) located near Asvestohori (coordinates 40^{o} 37′N, 23^{o} 44′ E). Six sampling sites were chosen in the vicinity each containing stands of *Quercus coccifera*, an evergreen schlerophyllous shrub. Site 1 (S1) was situated in the quarry. The other sites were located at distances and in directions from the quarry as follows: S2: 300 m, W; S3: 600 m, W; S4: 1.4 km, W; S5: 1.4 km, S; S6: 3 km SSE. S1-S4 were S-facing, S5 N-facing and S6 NNW-facing. S1-S5 had an elevation of 520 m and S6 605 m. Dust was not carried in a northerly direction mainly due to the prevailing winds and the barrier created by the quarry wall. To the east the land was used for agricultural purposes and did not contain stands of *Q. coccifera*.

Field measurements were carried out on six consecutive days in June 1993, between 9.00 and 10.00 am, using leaves from the SSE side of each of five bushes selected at random at each site. The same five bushes were used for all measurements. During this period the weather conditions were sunny and dry and almost identical on each day. Different leaves were used for measurements of

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photosynthesis and stomatal conductance, chlorophyll fluorescence and limestone dust deposition levels to avoid dust disturbance by air circulation in the IRGA chamber during photosynthesis and stomatal conductance measurements, or removal of dust by direct contact by PSM clips during chlorophyll fluorescence measurements.

Dust deposition at each site was determined using one leaf from each of the five different bushes selected at random. The amount of limestone dust on each leaf (upper and lower surfaces) was determined by rinsing each leaf in 10 ml of distilled H_2O in the field. The eluate was later digested with a nitric-perchloric acid solution (4:1 v/v) for 5 h at 150 °C and the concentration of Ca was determined in an atomic absorption spectrophotometer (Perkin Elmer 2380) using SpectrasoL (BDH Chemicals Ltd, Poole, England) as a standard solution. The Ca concentration was expressed as µg Ca cm^{-2} leaf area.

Photosynthesis and stomatal conductance of *Q. coccifera* were measured using a portable infra-red gas analyser (IRGA, LI-COR Model 6200, LI-COR, Lincoln, Nebraska) in a closed system, with a 0.25 L chamber. The rate of net CO_2 assimilation per unit leaf area (A) and stomatal conductance (g_s) were calculated using the equations of von Caemmerer and Farquhar (1981). Two leaflets, consisting of 3 to 4 leaves, were detached from each of the five bushes.

Chlorophyll fluorescence parameters (Fo, minimal fluorescence; Fm, maximal fluorescence; Fv, variable fluorescence where Fv=Fm-Fo; $t_{1/2}$, half-rise time from Fo to Fm) were measured *in situ* using a portable Plant Stress Meter (BioMonitor S.C.I. AB, Sweden) as described by Öquist and Wass (1988), on the upper surface of leaves which had previously been dark-adapted for 45 min. Chlorophyll was excited for 5 s by actinic light with a photon flux density of 400 µmol m^{-2} s^{-1}. Five leaves were selected from each of the five bushes.

Leaves were transported within 30 min to the laboratory for chlorophyll determination and leaf area measurements. Chlorophyll was determined on one of the leaves from each of the leaflets used in measurements of photosynthesis and stomatal conductance. Leaf discs (1 cm^2) were extracted in liquid nitrogen and incubated in 90% acetone for 24 h at -10 °C for complete extraction of chlorophyll. Total chlorophyll was determined from the absorbance at 664 nm and 647 nm and the extinction coefficients given in Jeffrey and Humphrey (1975). The leaf area of leaves used in the determination of limestone dust deposition levels, photosynthesis and stomatal conductance were measured using an Mk2 area meter (Delta-T Devices Ltd, UK) connected to a TC7000 Series Camera (Burle Industries Inc, USA).

Single regression analysis, followed by analysis of covariance (Sokal and Rohlf 1981), was used for testing the relationship between photosynthetic rate and stomatal conductance and the Ca concentration on leaves of *Q. coccifera*. Multiple regression (Sokal and Rohlf 1981) was used for assessing the relative contribution of photosynthetically active radiation (PAR), relative humidity in the leaf chamber and leaf temperature to the variation in photosynthesis.

RESULTS AND DISCUSSION

The average levels of deposition of limestone dust on leaves of *Q. coccifera* at each of six sampling sites, expressed as the Ca level per unit leaf area, were 2005.9, 120.2, 16.6, 20.9, 9.5 and 7.9 µg Ca cm^{-2} leaf area for S1-S6, respectively. Dust

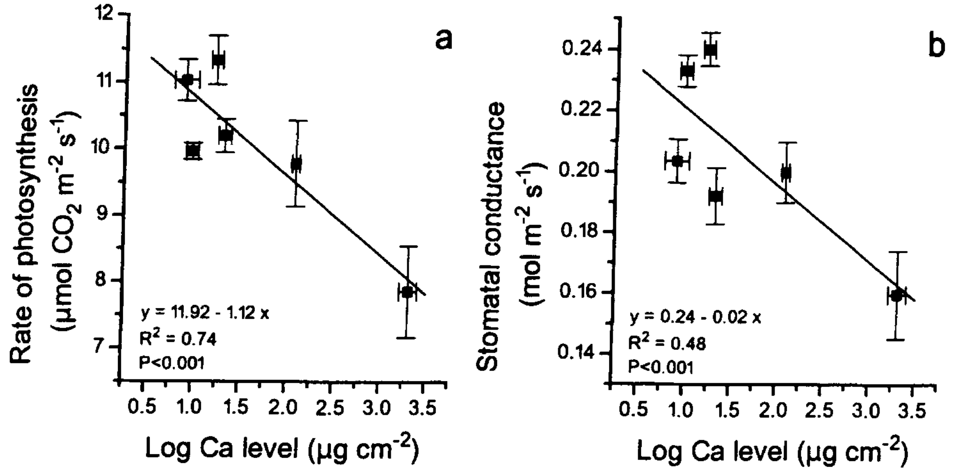

Figure 1. Relationship between the average level of limestone dust deposition on the leaves of *Quercus coccifera* per site, expressed as the logarithm of the Ca level µg cm^{-2} leaf area, (n = 5) and (a) the average rate of photosynthesis per site (n = 10) and (b) average stomatal conductance per site (n = 10). Vertical and horizontal bars represent standard errors.

deposition, when studied, has been given either as a rate (g m^{-2} d^{-1}) (field determined or experimentally applied) or as a level (mg cm^{-2}) of deposition covering the leaves or concentration of particulates in the atmosphere (µg m^{-3}). Therefore the level of dust covering the leaves in this study (µg Ca cm^{-2}) is difficult to compare with values of dust deposition in the literature expressed as total dust weight per leaf area. However, the rate of dust deposition on vegetation in the vicinity of limestone quarries has been reported to be 14.2 g m^{-2} d^{-1} (Manning 1971; Brandt and Rhoades 1972; 1973), but levels of deposition vary greatly (see Farmer, 1993).

The average rate of photosynthesis of *Q. coccifera* leaves at each site decreased exponentially with increasing levels of limestone dust on the surface of the leaves (Fig. 1a). The rate of photosynthesis was about 11 µmol CO_2 m^{-2} s^{-1} leaf area at the lowest levels of limestone dust deposition and about 8 µmol CO_2 m^{-2} s^{-1} at the highest. A similar decrease in stomatal conductance was also associated with increasing dust deposition on leaves of *Q. coccifera* (Fig. 1b). Using multiple regression the relative humidity, the photosynthetically active radiation (PAR) and leaf temperature in the leaf chamber contributed to only 7.95% of the variation in photosynthesis. Therefore, the changes in photosynthesis and stomatal conductance did not appear to be affected by other environmental factors other than the limestone dust. The decrease in the average photosynthetic rate per site as a result of increasing limestone dust deposition occurred faster than the respective decline in stomatal conductance. Analysis of covariance indicated that the slopes of the regression lines were significantly different ($P < 0.05$) suggesting that the reduction in stomatal conductance may not be the only factor responsible for the decline in photosynthesis. Since limestone quarry dust is highly alkaline, the caustic effect of the dust on the leaf surface may also be a factor. The reduced rate of photosynthesis and reduced stomatal conductance would be consistent with the blockage of leaf stomata by dust particles. Reduced photosynthesis and blocked stomata, amongst other effects, have been reported for some commercial

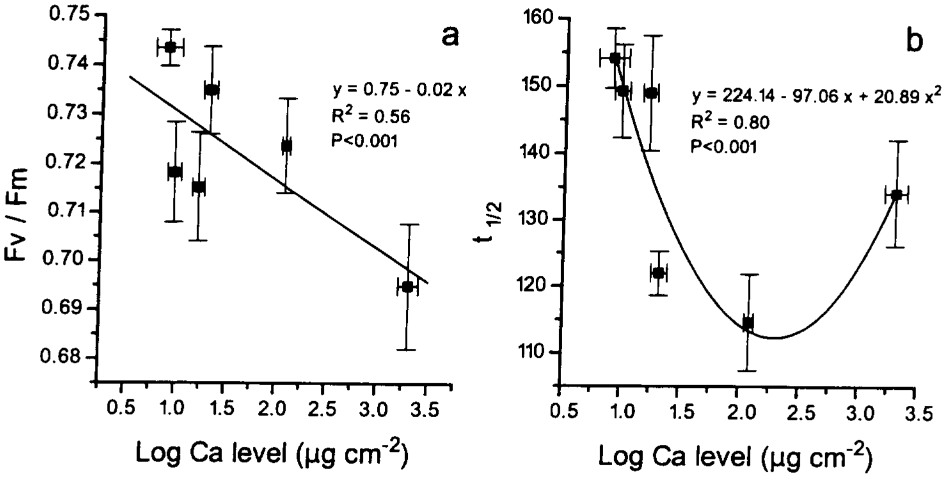

Figure 2. Relationship between the average level of limestone dust deposition on the leaves of *Quercus coccifera* per site, expressed as the logarithm of the Ca level μg cm^{-2} leaf area, (n=5) and (a) the ratio of the variable to maximal fluorescence, Fv/Fm per site (n=25) and (b) the half-rise time from Fo to Fm, $t_{1/2}$ per site (n=25). Vertical and horizontal bars represent standard errors.

herbaceous and fruit crops as a result of cement dust deposition on leaves (Darley 1966; Oblisami et al. 1978; Borka 1980). Blocked stomata and reduced diffusive resistance have also been reported as a result of urban road dust (Fluckiger et al. 1977; 1982), motor vehicle exhaust (Thompson et al. 1984) and dust from a smokeless fuel factory (Ricks and Williams 1974; 1975). In the studies by Ricks and Williams on *Quercus petroea*, leaf senescence and chlorophyll degradation were also promoted and the uptake of SO_2 enhanced.

In other studies, limestone dust has been reported to cause chlorotic needles in *Tsuga canadensis* (Manning 1971), reduced growth, with necrosis of the leaves and peeling bark, in the cases of *Acer rubrum*, *Quercus prunus* and *Q. rubra*, while increasing the growth of *Liriodendrum tulipifera* (Brandt and Rhoades 1972; 1973). Limestone dust has also been shown to affect the phylloplane flora of woodland species (Manning 1971) and cause changes in the community structure of natural woodland (Brandt and Rhoades 1972), grasslands and heathlands (see Farmer 1993) and epiphytic lichen communities (Gilbert 1976).

Chlorophyll fluorescence measurements particularly the ratio of Fv/Fm have been used as indicators of plant stress caused by a range of environmental factors (for example, Björkman and Demmig 1987; Bolhàr-Nordenkampf et al. 1989). Typically Fv/Fm decreases with increasing stress and reflects the reduced efficiency of PSII photochemistry (Krause and Weis 1991). The average value of Fv/Fm per site was observed to decrease with increasing dust deposition on the surface of *Q. coccifera* leaves (Fig. 2a) and Fv/Fm differed significantly between the lowest and highest dust levels (ANOVA, $P<0.001$). This is indicative that the efficiency of the primary photochemistry of PSII is reduced in leaves experiencing stress from limestone dust. Although a statistically significant exponential decrease of the average value of Fv/Fm per site is observed with increasing limestone dust deposition (Fig 2a), it is only the value of Fv/Fm at the highest dust deposition level that is responsible for this relationship. Analysis of the average Fv/Fm values at the lower dust deposition levels would indicate that these vary independently of dust level. For physiological, non-stressed plants from a wide range of species Fv/Fm ranges from 0.778-0.860 with a mean of 0.832(+/-0.004) (Björkman and Demmig 1987). All the values of Fv/Fm measured for *Q. coccifera* were lower than this range. The half-time for the rise from Fo to Fm, $t_{1/2}$, is taken as a measure of the size of the electron acceptor pool on the reducing side of PSII (see Krause and Weis 1991) which can be diminished by various stress treatments, such as ozone (Barnes et al. 1990) and Cd (Krupa et al. 1992). The average values of $t_{1/2}$ per site decreased with increasing dust deposition on the surface of the leaves with the exception of the highest dust level which was of an intermediary value (Fig. 2b). Generally this trend suggests that the size of the pool of electron acceptors on the reducing side of PSII was reduced as a result of increasing stress from limestone dust.

Average total chlorophyll (a+b) concentrations at each site ranged from 26.5-29.7 $\mu g\ cm^{-2}$ leaf area. The mean chlorophyll concentrations did not vary significantly between the sampling sites (ANOVA, $P>0.05$), suggesting that the leaves were not suffering seriously from the shading effects of the dust layer. Manning (1971) found that leaves of *Vitis vinifera* were a much darker green when exposed to limestone dust, which was probably a direct response to the shading effect of the dust. In contrast limestone dust caused chlorosis in *Tsuga canadensis* (Manning 1971) and chlorophyll degradation in the lichen *Physcia adscendens* (Zaharopoulou et al. 1993).

In conclusion, the results indicate that the deposition of limestone dust on the surface of *Q. coccifera* leaves was detrimental to photosynthesis. This could be partially explained in terms of reduced stomatal conductance, that is the blockage or closure of stomata, but may also be due to the direct chemical effects of the dust on the leaves, for example the alkaline pH of the dust. That leaves were stressed was also observed by the decrease in Fv/Fm indicating a reduction in the efficiency of the photochemistry of PSII and by a decrease in $t_{1/2}$ suggesting a diminished electron acceptor pool on the reducing side of PSII.

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