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(Evapo)Transpiration Measurements Over Vegetated Surfaces as a Key Tool to Assess the Potential Damages of Air Gaseous Pollutant for Plants

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1. Introduction

The term evapotranspiration (ET) is used to describe the contemporary evaporation of water from surfaces and transpiration of water through stomata.

Evaporation (E) consists in the change of state of water from liquid to vapour that occurs when water molecules momentarily acquire high speed near the surface of water as the result of collisions with other molecules. This kind of process can be enhanced by environmental factors, such as direct solar radiation and temperature, which provide the required energy.

Leaf transpiration can be thought of as a necessary cost associated with the opening of stomata to allow the diffusion of carbon dioxide inside the leaf, for photosynthesis and plant growth. However, these low-resistance apertures also provide a favourable diffusional pathway for atmospheric gas pollutants, such as ozone, that can reach the substomatal cavity and the mesophyll inside the leaf, (causing negative effects at different biological and physiological levels).

Stomata opening is regulated by environmental factors such as light, air temperature and humidity, wind speed and water availability in the soil (Jarvis, 1976; Stewart, 1988).

The interpretation of the ET and the understanding of the potential influencing factors are important topics for ecophysiology, agriculture and agro-meteorology since the past century (Penman, 1948).

ET plays a key role in the water cycle, affecting the water balance from local up to regional scale and causing feedback between vegetation and climate (Wilske et al. 2010). Penman (1948), combining the energy balance with the mass transfer method, derived an equation to calculate the evaporation from an open water surface using meteorological data such as radiation, temperature, humidity and wind speed. Today the Penman-Monteith equation is considered the most reliable method to calculate evapotranspiration.

The FAO (Food and Agriculture Organization of the United Nations) Penman-Monteith equation states that evapotranspiration can be obtained as it follows:

$$\lambda E = \frac{(R_N - G)s + \frac{c_p \rho [e_s(T_a) - e]}{r_a}}{s + \gamma \left(\frac{r_a + r_s}{r_a} \right)} \quad (1)$$

where R_N is the net radiation, G is the soil heat flux, $(e_s - e_a)$ represents the vapour pressure deficit of the air, ρ is the mean air density at constant pressure, c_p is the specific heat of the air, s represents the slope of the saturation vapour pressure temperature relationship, γ is the psychrometric constant, and r_s and r_a are the surface and aerodynamic resistances.

The Penman-Monteith approach as formulated above includes all parameters that govern energy exchange and corresponding latent heat flux (evapotranspiration) from ecosystems. Slightly different formulations and simplification of this equation have been proposed and used for different purposes.

In this chapter it will be showed how it is possible, starting from transpiration measurements at leaf level, at plant level or at ecosystem level, to estimate how much ozone is taken up by the vegetation through the stomata. Ozone is in fact widely recognized to be an air pollutant which may cause significant damages to the vegetation. At leaf level ozone can damage mesophyll cells, affecting the photosynthetic activity and causing the appearance of chlorosis and necrosis. This lead the plant to spend energy for the detoxification, causing, in turn, a decrease of the available energy for the plant growth. As a consequence, stem and root dimension, stem and root ratio, crown architecture and productivity can be influenced by ozone uptake (Musselman et al. 2006). A key passage for estimating the ozone uptake at each scale (leaf, plant or ecosystem level) is the determination of the stomatal conductance.

2. Measuring techniques of stomatal conductance

Evapotranspiration from an ecosystem is influenced by physiological and morphological properties of the canopy (Baldocchi & Vogel, 1996), and soil properties that govern the water vapor exchange at the soil-atmosphere interface (Baldocchi et al., 2000). Additionally, meteorological conditions within and above the canopy, such as wind speed, air temperature and humidity, are further influencing factors that must be taken into account. Different methodologies are available to obtain indirect measurements of stomatal conductance, all of them are linked to transpiration measurements with the same factors influencing both of them.

2.1 Leaf level measurements

Different instruments, such as porometers and gas exchange devices, allow to measure stomatal conductance at leaf level taking into account the environmental parameters (temperature, relative humidity, solar radiation) inside the measuring cuvette.

Three main types of porometers are available: steady-state porometers, dynamic porometers and null balance porometers.

Steady-state porometers (e.g. Leaf porometer, Decagon Devices, Pullman, USA) are based on the measurement of leaf conductance in series with two elements whose conductance is

known, using a sensor head with a fixed diffusion path. Since the conductance of the sensor elements are known, assuming that the leaf tissues are at a saturation point and that leaf temperature is equal to the air temperature of the nearer sensor, the leaf conductance can be calculated applying the Ohm's law and by measuring air temperature and relative humidity in two points.

The principle of operation of a null balance porometer (e.g. LI-1600, LI-COR, Lincoln, USA) is to manually maintain the air relative humidity at a set-point inside the cuvette. The transpiration from a leaf is obtained by measuring the flow rate of dry air which is necessary to keep constant the relative humidity inside the cuvette where the leaf is placed. Usually the environmental relative humidity of the air is assumed as a null point. The stomatal conductance is hence calculated from the measurements of relative humidity, leaf and air temperature and flow rate of dry air.

Dynamic porometers (e.g. AP4, Delta-T Devices LTD, Cambridge, UK) measure water stomatal conductance indirectly. In fact stomatal conductance is derived from the measurement of the time which is needed to the leaf to release inside the cuvette enough water vapour so that the relative humidity inside the cuvette increase by a fixed amount (2.3%). As a matter of fact, the measurement is constituted by a cycle of measurements to warrant reproducibility. The stomatal conductance is finally obtained by comparing the measurement with a calibration plate whose conductance is known (Monteith et al., 1988; Bragg et al., 2004).

Adding other environmental measurements like soil water content it is possible to apply a Jarvis-type model (1976) to estimate stomatal conductance, further developed by Stewart (1988).

The Jarvis-Stewart model hypothesize that the stomatal conductance of vegetation can be modelled as the product between the maximum stomatal conductance and a series of limiting functions whose values are between 0 and 1. These functions depend on environmental parameters such as air temperature, VPD, soil water content (or soil water potential) and light (PAR or global radiation).

In details the model foresee stomatal conductance g_s as:

$$g_s = g_{\max} \cdot [f_T \cdot f_{\text{light}} \cdot f_{\text{VPD}} \cdot f_{\text{SWC}}] \quad (2)$$

where g_{\max} is the highest stomatal conductance of the studied vegetation, this is a value determined by the genetic characteristics of the species, it is often measured in laboratory experiment letting the studied species grow in optimal conditions; f_T , f_{light} , f_{VPD} and f_{SWC} are the limiting functions of temperature, PAR, VPD and soil water content respectively.

The first step in order to obtain these functions is to perform measurements of stomatal conductance at leaf level, by means of porometers or gas exchange analyzers, covering a large scale of different conditions for each environmental factor; then the relative stomatal conductance (g_s/g_{\max}) is plotted versus the corresponding values of each variables. Finally the function is obtained by a boundary-line analysis of the plot. Leaf-level measurements should be performed in largely different conditions (T, PAR, VPD and SWC) in order to define the most representative model.

The Jarvis-Stewart approach is widely used especially in the UN/ECE (United Nations-Economic Commission for Europe) scientific community in order to calculate the ozone stomatal dose absorbed by plants. Recently the ICP Vegetation (The International

Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops) proposed some modifications of the original Jarvis-Stewart model introducing new standard limiting functions (ICP Vegetation, 2004)

First of all, leaf stomatal conductance is modelled as it follows:

$$g_s = g_{\max} \cdot [\min(f_{O_3}, f_{PHEN})] \cdot f_{light} \cdot \max[f_{\min} \cdot (f_T \cdot f_{VPD} \cdot f_{SWC})] \quad (3)$$

where g_{\max} is the maximum stomatal conductance of the studied specie and f_{O_3} , f_{PHEN} , f_T , f_{light} , f_{VPD} and f_{SWC} are the limiting functions depending on ozone concentration, phenology, temperature, PAR, VPD and soil water content respectively. Function f_{\min} represents a threshold value for the functions of the temperature, VDP and SWP. The part of the Eq. 3 related to f_{PHEN} and f_{O_3} is a most limiting factor approach. Either senescence due to normal ageing is limiting or the premature senescence induced by ozone is limiting. Early in the growing season and at low ozone exposure f_{PHEN} is always limiting and f_{O_3} then does not come into operation (ICP Modelling and mapping, 2004). Furthermore, it is worth to be noticed that the limiting function f_{O_3} has been calculated only for potato and wheat.

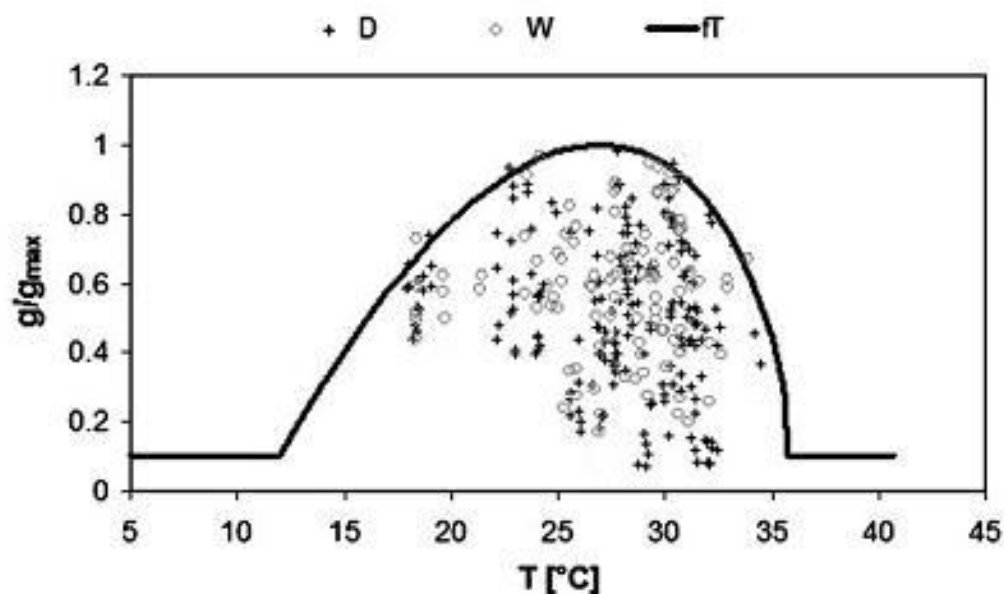


Fig. 1. Limiting function f_T for the stomatal conductance model of poplar. g/g_{\max} is the relative stomatal conductance to water vapour while T is the temperature. D are measurements on non-watered plants while W are measurements on watered plants (modified from Gerosa et al., 2008).

As an example, the limiting function for the temperature adopted by Gerosa et al. 2008 in an Open-Top Chambers experiment is shown in Figure 1. The f_T function is described by:

$$f_T = \frac{(T - T_{\min})}{(T_{opt} - T_{\min})} \cdot \left[\frac{(T_{\max} - T)}{(T_{\max} - T_{opt})} \right]^b \quad (4)$$

where T represents the temperature of the air near the leaf; T_{opt} (27 °C), T_{\max} (36 °C), T_{\min} (11 °C) are 3 species-specific parameters, respectively representing optimal, maximum and minimum temperatures and b (adimensional) is defined as:

$$b = \frac{(T_{\max} - T_{opt})}{(T_{opt} - T_{\min})} \quad (5)$$

If T is greater than T_{\max} or smaller than T_{\min} , the function f_T is given the minimum value of 0.1.

In a similar way it is possible to obtain the other limiting functions and hence to estimate the stomatal conductance to water and the stomatal conductance to ozone by multiplying the former by 1.68, which represents the ratio between the diffusivity of water in air and the diffusivity of ozone in air.

The ozone stomatal dose is finally obtained as the integral of F_{ST} over the measuring period

$$D_{ab} = \int_{t_a}^{t_b} F_{ST}(t) dt$$

Further details about the intermediate passages and results can be found in Gerosa et al. (2008).

Leaf level measurements of stomatal conductance are a useful tool for studying physiological behaviour of vegetation both in natural and controlled environment. On the other hand, the importance of studying vegetation behaviour at ecosystem level is fundamental for many research fields such as climatology, hydrology and air quality. Hence, upscaling forest ecological processes and their link with canopy structure has puzzled researchers from different fields (Ewers *et al.*, 2007; De Pury & Farquhar, 1997; Cescatti & Zorer, 2003; Martens *et al.*, 2000; Law *et al.*, 2001). The development of a reliable upscaling method would permit the evaluation of important ecosystem functions such as: gross primary productivity (GPP), evapotranspiration, stomatal uptake of air pollutants, biogenic volatile organic compounds (BVOCs) emissions.

Upscaling conceptually consists of the integration of the physiological characteristics (for instance stomatal conductance or transpiration) of all the elements in which the canopy is divided. (Van de Zande et al., 2009). These physiological characteristics are controlled by the environmental factors surrounding that element and light is usually the most varying factor in space and time. The concept of upscaling is fundamental for the bottom-up approach and two main problems can be identified:

- the description of the light environment inside the canopy
- how physiological characteristics scale with that light environment.

Knowing that the light environment inside a canopy is the resultant of the interaction of the canopy structure with the incoming light, a full description of the light environment requires high detailed knowledge of the canopy structure. Canopy structure can be defined as the temporal and spatial organization of the above ground vegetation components including their position, extent, quantity, type, orientation, shape, and connectivity (Parker, 1995).

One of the main assumptions about light penetration is that the canopy is often considered as a turbid medium in order to simplify the light-vegetation interaction. In this way it is possible to apply the Lambert-Beer's law (Monsi and Saeki, 1953) to describe radiation attenuation in the canopy. In a canopy, the average (temporal and spatial) distribution of light shows a profile which decline with the height. This behaviour is well modelled by the Lambert-Beer's law. On the contrary instantaneous profiles are not well modelled.

Furthermore, the leaf angle is fundamental in determining the absorbed irradiance in canopies: top-canopy leaves could absorb less light from direct irradiation than leaves inside the canopy (De Pury and Farquhar, 1997).

Different upscaling approaches have been used in literature: the big-leaf model, two leaves model and multilayer model.

The widely used big-leaf approach considers the whole canopy equivalent to a single big-leaf. This approach allows a reliable upscaling, but only after parameterization over reference data. In fact one of the main disadvantages of this approach is that the model for different levels of irradiance through the canopy is solved by applying semi-empirical corrections for the canopy photosynthetic capacity. Furthermore these corrections may depend on environmental conditions or crown architecture, thus reducing the application of the model in different conditions. Despite this limitations, big-leaf models have been shown to well describe canopy photosynthesis (Amthor et al. 1994; Lloyd *et al.* 1995) and thanks to their limited number of input, big-leaf models were employed for large scale modelling.

Norman (1993) and Raupach (1995) pointed out that big-leaf models do not apply a real scaling up procedure: the variables involved at leaf level have different relationships among them at canopy level and hence canopy dynamics may be misinterpreted in terms of the leaf level behaviour.

Interesting results were obtained by De Pury and Farquhar (1997): they proposed to split the big-leaf into sunlit and shade fractions, which are subject to changing during the day. Canopy irradiance absorption and photosynthetic capacity change during the day because of the variation of sunlit and shade leaves fractions. The significant improvement of this model is the simultaneous use of five different features that were already used separately in models. These features are: distinction between sun and shade leaves; change of photosynthetic capacity through the canopy; use of photosynthetic biochemical modelling; a temporal dependant partitioning between sun and shade leaves; a single layer model with relatively simple calculations. But probably, the most important feature of this model is its scaling approach that allows a direct scaling of parameters from leaf to canopy scale.

Before the introduction of the sun/shade model by De Pury and Farquhar (1997), multi-layer models were already used (e.g. Norman, 1982). In these models the canopy is split into multiple layers and each layer is usually separated into sunlit and shaded fractions, and the sunlit fraction is divided into different leaf angle classes. Absorbed irradiance is calculated for the shaded fraction and for each leaf class of the sunlit fractions separately. Vertical profile of leaf nitrogen and of photosynthetic capacity are well modelled by this approach but the number of required input is very large. De Pury and Farquhar (1997) compared first a big-leaf model and a multi-layer model with measurements run at Wagga Wagga, Australia (de Pury, 1995). Despite the previous mentioned fine tuning of the big-leaf model better results were obtained from the multi-layer model. The most significant result by De Pury and Farquhar (1997) was the good agreement between their sun/shade model and the multi-layer models and measurements.

2.2 Plant level measurements

Sap is the fluid flowing through the xylem of plants, and it is constituted by water and nutrients brought up from the roots through the plant and finally to the leaves from where most of it is evaporated. The flow of sap is, hence, more or less equivalent to the whole plant transpiration. The water transport through the xylem is not realized by energy spent by

tracheary elements, which are dead, but by a passive mechanism due to the difference in water potential between the leaves and the soil.

The most important cause of xylem sap flow is the evaporation of water from the surfaces of mesophyll cells to the atmosphere. Liquid water reaches the transpiring surface through nanopores in the mesophyll cell walls and, when transpiration occurs, water forms menisci in each of the nanopores with a curvature depending mostly on the transpiration rate. Transpirational pull results from the evaporation of water from the surfaces of cells in the leaves. This evaporation causes the surface of the water to recess into the pores of the cell wall. By capillary action, the water forms concave menisci inside the pores. The high surface tension of water pulls the concavity outwards, generating enough force to lift water as high as a hundred meters from ground level to the highest branches of trees.

The resulting surface tension causes a negative pressure (or tension) in the xylem that pulls the water from the roots and soil. Sap flow hence occurs when the pressure in the leaves is lower than that of the soil. (Tyree, 1997).

In some circumstances (dry soil and fog for example), leaf water potential can be less negative than that of roots, and in this case a reverse flow can occur (Burgess et al, 2004). If the water potential of the root cells is more negative than that of the soil, usually due to high concentrations of solute, water can still move by osmosis into the root from the soil. This causes a positive pressure that forces sap up the xylem towards the leaves. This phenomenon, guttation, however is not usually observed in woody species.

Transpirational pull requires that the vessels transporting the water are very small in diameter, otherwise cavitation would break the water column. When the water pressure within the xylem reaches an extreme tension due to low water input from the roots (if, for example, the soil is dry and transpiration is high), gaseous species, dissolved in the xylem abandon the liquid, or are sucked in from near by tissues, forming an air bubble - an embolism forms and the vessel is no more functional. Although the mechanism has not been cleared yet, embolism can be cured by the plant possibly by an active mechanism of companion cells.

Methods for sap flow measurements, based on thermodynamic principles, are widely used in plant physiology and hydrology studies. They have many advantages compared to other techniques and they are easy to apply for long-term automated monitoring of water movement in plants. Detailed reviews of the most frequently applied methods (heat pulse velocity, HPV; tissue heat balance, THB; stem heat balance, SHB; heat dissipation, HD) have been presented in many papers (Pickard & Puccia, 1972; Pickard, 1973; Swanson & Whitehead, 1981; Swanson, 1983, 1994; Jones et al., 1988; Valancogne & Nasr, 1989; Campbell 1991; Groot & King, 1992; Barret et al., 1995; Grime et al., 1995; Edwards et al., 1996; Smith & Allen, 1996, Braun, 1997; Kostner et al., 1998; Wullschleger et al., 1998; Steppe et al., 2010). The theoretical background for heat flow in sapwood has been developed by Marshall (1958), 26 years after convected heat pulses were first applied as indicators of sap velocity in trees (Huber, 1932). Nevertheless, empirical works within that period (Baumgartner, 1934; Dixon, 1936; Kuniya, 1950) were important for understanding the complex physical processes of the heat transfer in living wood.

Essentially two big groups can be distinguished among thermal methods: those applying pulse heating and those based on continuous heating.

Measurements of sap flow, based on heat transfer methods, display two different arrangements of differential thermocouples around the heater: a symmetrical one, with

the ends of the thermocouple placed at equal distances below and above the heater along the axial direction (e.g. Daum 1967; Vieweg & Ziegler, 1960; Sakuratani, 1981; Nadezhdina et al., 1988) and an asymmetrical one with the upper end of the thermocouples placed at the same axial height of the heater and the lower (reference) end of the thermocouple placed at a certain distance below the heater (e.g. Ittner 1968; Cermak et al. 1973; Granier 1985).

The symmetrical arrangement of thermocouples was first applied as a modification of the heat pulse method, termed the heat ratio method, HRM. The background theory for HRM was suggested by Marshall (1958). Marshall derived an equation for the heat pulse velocity, V_h , as the ratio of the increase in temperature after releasing a heat pulse at points equidistant downstream and upstream from the heater:

$$V_h = \frac{k_{st} \times \ln(T_1/T_2)}{\rho_{st} \times cp_{st} \times X} \quad (6)$$

or

$$V_h = \frac{D_{st} \ln(T_1/T_2)}{X} \quad (7)$$

where, k_{st} , ρ_{st} , cp_{st} and D_{st} are thermal conductivity, density, specific heat capacity and thermal diffusivity of green stem wood; T_1 and T_2 are increases in temperatures at equidistant points X cm above and below from the heater. This arrangement of thermocouples allows the HRM method to measure low and reverse flow rates.

Vieweg and Ziegler (1960) were the first who applied continuous heating for sap flow measurements. They were also the first who spoke of a deformation of the heat field ("cloud" in their terminology) caused by the moving of sap. Using a series of thermometers, placed in the axial direction above and below the heater, they showed that when the sap velocity was zero, the heat field around the heater was symmetrical, while when the sap was moving, the heat field becomes asymmetrical. No equations were given by the authors approving the method quantitatively, but experimentally it was found that the system is very sensitive and that it can measure sap velocities down to 3 cm h⁻¹ in large larch and birch trees. Several decades later the infrared technology introduced by Anfodillo *et al.* (1992, 1993) and applied in further sap flow studies (Granier *et al.*, 1994; Nadezhdina et al., 2004) allowed to "see" *in situ* the deformation of heat field in live trees.

Vieweg and Ziegler (1960) suggested to take the extent of asymmetry of the heat field (and thus of the temperature differences in the xylem) as a measure of sap velocity. Ten years later, Saddler and Pitman (1970), revised the method of Vieweg and Ziegler (1960). Their novel contribution was to develop an analytical solution. The authors modified the equation of heat conduction in a woody stem solved by Marshall (1958) for the case of an instantaneous line source of heat released into an infinite block of wood substance. The solution of the authors was derived for steady-state conditions in an infinite uniform cylinder with uniform continuous heat supply within a cross-section. Their theoretical considerations showed that the logarithm of the ratio of the temperature difference ($T - T_a$) in the "downstream" position, T_+ to that in the "upstream" position, T_- gives a measure of the sap flux, u , (named by them as sap speed index, SSI) by the equation:

$$u = \frac{k_{st} \times \ln\left(\frac{T_+}{T_-}\right)}{\rho_{st} \times cp_{st} \times X} \quad (8)$$

where ρ_t and cp_{st} are density and specific heat of sap, respectively, k_{st} is the thermal conductivity of the stem and X is the axial distance between the heater and the thermocouples. In addition, the authors also mentioned that the values ρ_{st} and c_{st} could be taken as those for water without appreciable error because the composition of sap would be sufficiently close to pure water.

When comparing both approaches, suggested by Marshall (1958) for heat pulse (HRM) and by Saddler and Pitman (1970) for steady-state heat flow, it could be observed that the derived Eq. 6 and Eq.8 are very similar. The main difference relies on the measured temperature variables around the heater source. Such similarity is explained by the use of a “compensation” approach for heat movement through conduction by introducing equidistant points for temperature measurements from a heat source in both cases.

The majority of methods that apply symmetrical arrangements of thermocouples around a heater, have very high sensitivity and good relation with the true sap flow under low flow rates (Vieweg and Ziegler, 1960; Saddler and Pitman, 1970; Nadezhdina, 1999). However, the situation is different under high flow rates. The output from a symmetrical sensor gives a diurnal variation with two peaks, one in the morning and one in the afternoon, and a drop around midday (e.g., Ittner, 1968 in spruce, Nadezhdina, 1988, 1999 in apple).

The temperature difference between the heater (or the needles, spaced in the middle of heated elements) and the reference needles, which measure the sapwood ambient temperature below the heater, is used for the sap flow calculation in both tissue heat balance, THB, and heat dissipation, HD, methods. This temperature difference is termed as asymmetrical, dT_{as} .

The THB method was developed for sap flow measurements in large trees in the late 1970th (Čermák et al., 1973; Kučera et al., 1977) and it is commercially available and rather widely used in field experiments.

For trees with stem diameter over 70 mm Granier (1985) described the continuously heated probe technique based on empirical calibration (HD technique). Thanks to its simplicity and robustness, this technique became one of the most used for sap flow measurements in the field.

However, it is problematic to measure extremely low flow rates using these methods. There are also problems to distinguish accurately between zero flow and fictitious flow (or a similar variable corresponding to the heat losses from the segment), which must be subtracted from the recorded data in order to get the true sap flow (Burgess et al., 2000). Do & Rocheteau (2002a, 2002b) underlined, that for the HD-method, zero flow must be assumed during certain nights and further external information (e.g., the natural defoliation) should be used to test the assumption. Both the THB and the HD methods cannot measure bi-directional flows, although possibility to use the modified thermal dissipation technique for bi-directional measurements in roots was reported more recently by Brooks *et al.* (2002).

In spite of the fact that numerous works improving the theory and instrumentation for thermodynamic methods have been done since the first application of heat as an indicator for sap movement in trees, there is still a lot to do in order to decrease limitations for the methods and instrument application and to increase their accuracy. Some of the problems cannot be avoided by nature (e.g. circumferential and radial variation of flow in trees),

others we have to cope with (size of trees, wound effect, sensitivity under low rates, bi-lateral flow records, high time resolution, probe spacing, natural temperature gradients, variable water content, etc.).

In 1991, while working with the “sap flow index”, Nadezhdina focused on a new method based on the heat field deformation (HFD) (Nadezhdina, 1989; 1992; 1999). This method is capable to measure over a wide range of flow rates without losing the advantages of other methods based on a continuous heating, i.e. high time resolution. Since 1996, the method was further developed (Nadezhdina & Čermák, 2000; Nadezhdina *et al.*, 1998) and it was intensively tested in different species (up to 50) and different environmental conditions. The fundamental idea was to combine the advantages of the earlier methods based on the measurements of asymmetrical and symmetrical temperature gradients, dT_{sym} and dT_{as} , around a heat source and, hence, to avoid the related limitations of the methods, when applying each of them separately. Data of temperature gradients, measured simultaneously by differential thermocouples at different distances around a heater (Nadezhdina, 1998) or calculated from the absolute temperature grid around the heater (Nadezhdina *et al.*, 1998), were used to characterize the shape of the heat field around it. Then measured temperature gradients were compared against SFI (or dT_{sym}) and sap velocity, measured by HPV method, and theoretical heat field shapes corresponding to different flow rates were reconstructed (Nadezhdina, 1998). This way, also the non-linear relationship between SFI and sap velocity as well as the sensitivity of SFI for measurements of very low rates, including zero and reverse flow, were explained.

It is assumed that the deformation of the heat field is caused by the real sap flow rate (and not by the heat velocity), in fact, heat dissipation is due to the flux and not to the velocity of the cooling medium.

The sap flow density (Q) is then scaled by sapwood / leaf area ratio (LA/SA) resulting equal to transpiration per unit leaf area, G_s can finally be derived from sap flow measurements, based on a simplification of the Penman-Monteith equation (Whitehead and Jarvis, 1981; Pataki *et al.*, 1998; Martinez-Vilalta *et al.*, 2003):

$$G_s = \frac{\gamma \times \lambda \times Q_l}{\rho \times c_p \times VPD} \quad (9)$$

where γ is the psychrometric constant (kPa K^{-1}), λ is the latent heat of vaporization of water (J kg^{-1}), ρ is the density of air (kg m^{-3}), c_p is the specific heat of air at constant pressure ($\text{J kg}^{-1} \text{K}^{-1}$) and $VPD = [e_{sat}(T) - e(T)]$ is the vapour pressure deficit (kPa) of the air. The simplification can be considered valid if G_s is predominant over the leaf boundary layer conductance, g_b (Whitehead and Jarvis 1981) i.e. when the canopy is strongly coupled with the atmosphere.

The ozone stomatal dose can be obtained as the integral of the product between G_s and the ozone concentration at canopy level (C_C):

$$D_{ab} = \int_{t_a}^{t_b} G_{ST}(t) C_C(t) dt \quad (10)$$

2.3 Ecosystem level measurements

Eddy covariance (EC) technique is the most direct technique, among the ones which have been developed through the last decades. in order to measure and calculate fluxes of a scalar

over a surface. This technique was applied for the first time by Swinbank (1951) and only recently was largely used and it is considered as a reference method because of the absence of assumptions for its applicability.

This technique is based on the simultaneous measurement of the fluctuations both of the vertical component of the wind (w) and of the considered scalar whose the fluxes are measured, i.e. specific humidity for evapotranspiration and latent heat fluxes, air temperature for sensible heat fluxes, ozone concentration for ozone fluxes (Fowler & Duyzer, 1989). Vertical fluxes are obtained as the average of the product between the fluctuations of w and the fluctuations of the studied variable from their average values, i.e. their covariance.

EC technique requires fast-response instrumentation in order to measure the vertical component of the wind (ultrasonic anemometer) and the studied scalars (hygrometer and fast response analyzers) (Hicks et al., 1989). These instruments must have a response time less than 0.1 s in order to be able to measure all the spectrum of the vortices which drive the flux and hence avoid the so called flux-loss and the subsequent empirical corrections (Moncrieff et al., 1997; Garratt, 1975). EC was first adopted only for short field campaigns (1/2 weeks) but since some years it has been widely employed in permanent monitoring field stations.

At ecosystem level, stomatal conductance, and hence ozone stomatal fluxes, the dose taken up by the ecosystem, cannot be measured directly, but only calculated by means of the so called SVAT (Soil-Vegetation-Atmosphere-Transfer models) models. Even if there are differences in the parameterizations adopted by these models, they are all based on an electric analogy in the flux description (Monteith and Unsworth, 1990; Garratt, 1994). Ozone flux is equivalent to a current intensity, ozone concentration difference is equivalent to a voltage difference and the resistance to the ozone diffusion through the atmosphere are parameterized by means of micrometeorological variables. This analogy was first used by Chadwick and Chamberlan in 1953.

One of the models that uses this analogy is the big-leaf model. It must be noticed that the big-leaf model described in this section is used with different purposes: in the section about leaf level measurements big-leaf model is used in a prognostic way, that is from leaf level measurement the model foresees the whole canopy behaviour (a bottom-up approach), while hereafter the big-leaf model is used in a diagnostic way, that is stomatal conductance is inferred from measurements of quantities referred to the whole canopy (a top-down approach). In this case, the big-leaf model states that, concerning vertical exchange of energy and matter, the whole canopy of an ecosystem can be assumed to be equivalent to a big-leaf at the displacement height $z=d+z_{0X}$ above the soil, where d is the zero plane height ($\approx 2/3$ of canopy height) and z_{0X} is the roughness length for the X (momentum, heat, concentration of a gas) scalar.

The displacement height can change if fluxes of momentum or sensible heat or latent heat or matter are considered: in fact $d+z_0$ is the height where momentum become null inside the canopy, which usually differs from the height from where there is the source for sensible and latent heat or from the height where ozone concentration is null ($d+z_{0O_3}$). Because of the similarity of the diffusion processes in Atmospheric Surface Layer (Monin and Obukhov, 1948) $d+z_{0O_3}$ is usually set equal to $d+z_{0H}$ and simply indicated as $d+z_0$. Furthermore, above the surface there is a thin sub-laminar layer. where the transport is no longer due to turbulence.

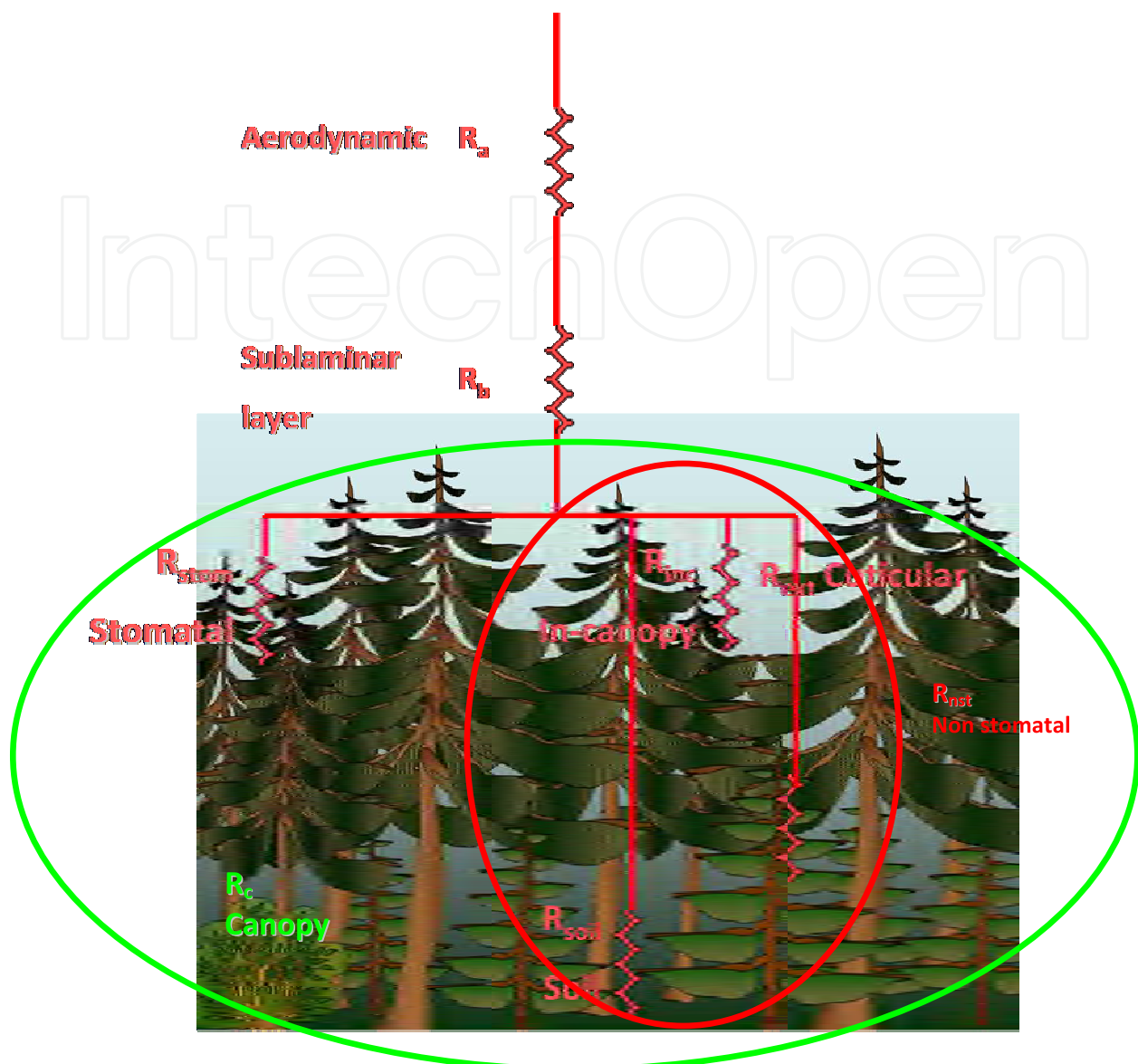


Fig. 2. Resistive scheme adopted: the aerodynamic resistance R_a , the sublaminar layer resistance R_b and the canopy resistance R_c . R_c is decomposed into two resistances (included in the green oval line): the stomatal resistance R_{stom} and the non stomatal resistance R_{nst} . The in-canopy resistance R_{inc} , the soil resistance R_{soil} and the cuticular resistance R_{ext} are some of the possible pathways in which the non stomatal deposition can be decomposed. In the big-leaf model described here these three resistances are not considered.

The total resistance to the ozone diffusion is equal to three series resistances:

$$R_{tot}(z) = R_a(d + z_0, z) + R_b + R_c \quad (11)$$

where R_a and R_b are atmospheric resistances and R_c is the integrated resistance of the exchange surface.

$R_a(d + z_0, z)$ is the aerodynamic resistance that ozone (but also any other scalar quantity) faces during the turbulent transport from the height z to the height $d + z_0$ (momentum sink). This

resistance mirrors wind behaviour and thermodynamic characteristics of the atmosphere such as the stability. Dyer (1974) proposed for R_a the following formulation:

$$R_a = \int_{d+z_0}^{z_m} \frac{\Phi_H(\zeta)}{k u^* z} dz \quad (12)$$

where: $\Phi_H(\zeta) = [1 + 5\zeta]$ if $\zeta = (z - d)/L > 0$

$$\Phi_H(\zeta) = [1 - 16\zeta]^{-1/2} \text{ if } \zeta \leq 0$$

k is the von Karman constant, u^* is the friction velocity and L is the Monin-Obukhov length. R_b is the resistance faced by ozone through the sub-laminar layer and it depends on ozone molecular diffusivity in the air and it is specific for the considered gas. Hicks et al. (1987) suggested for R_b :

$$R_b = \frac{2}{k u^*} (Sc / Pr)^{2/3} \quad (13)$$

where k is the von Karman constant, u^* is the friction velocity, Sc is the Schmidt number for the ozone ($\cong 1.07$) and Pr is the Prandtl number for the ozone ($\cong 0.72$).

R_c represents the surface or canopy resistance to ozone deposition and it includes all the processes linked to the influence of the system plant-soil on vertical exchange of the gas (e.g. stomatal uptake, deposition on non transpiring surfaces).

R_c strongly depends on the physiological characteristic of the vegetation (e.g. stomatal conductance), on the phenological characteristics of the vegetation (e.g. crown architecture, LAI) and on the underlying type of soil. R_c values are one or even two orders of magnitude greater than R_a and R_b , highlighting the great importance of the role played by vegetation in the deposition process. The relatively less importance of the two other resistances explains why even using different parameterizations of R_a and R_b , some authors obtains similar results. The equivalent total resistance can be obtained as the ratio between the total ozone fluxes and the ozone concentrations at the measuring point. This latter term is as a matter of fact the difference between the ozone concentration at the measuring point and the ozone concentration in the substomatal cavity, but this is usually assumed null as proposed by Laisk (1989). R_c can be finally obtained as the residual between R_{tot} and R_a plus R_b .

R_c is hence considered equivalent to two parallel resistances:

$$R_c^{-1} = R_{ST}^{-1} + R_{NS}^{-1} \quad (14)$$

where R_{ST} is the stomatal resistance and R_{NS} which includes all the pathways of destruction of ozone such as deposition on non transpiring surfaces, on the soil or by means of chemical reactions. All these pathways are collectively named non-stomatal resistance (Cieslik, 2004). R_{ST} could be deduced from the Penman-Monteith equation (Monteith, 1981), where the latent heat flux (that is proportional to the evapotranspiration). The Penman-Monteith approach attributes the whole evaporative process to the stomatal activity and thus it is effective only if E from the soil is negligible. This condition is satisfied if the soil surface is dry or the canopy of the studied ecosystem is completely close (Biftu & Gan, 2000). In any case Choudhury e Monteith (1988) showed that evaporation from the soil is always less than 5% if the ecosystem LAI is greater than 1. R_s is obtained as:

$$\lambda E = \frac{(R_n - G) \cdot \frac{\partial e_s(T)}{\partial T} + \frac{\rho c_p [e_s(T) - e]}{R_a + R_b}}{\frac{\partial e_s(T)}{\partial T} + \gamma \left(1 + \frac{R_s}{R_a + R_b} \right)} \quad (15)$$

where R_n is the net radiation flux, G is the soil heat flux (both are expressed in W m^{-2}); c_p is the air specific heat capacity ($\text{J K}^{-1} \text{Kg}^{-1}$) and c_p is air density (kg m^{-3}); e and $e_s(T)$ are respectively water vapour pressure and water vapour pressure at saturation (both Pa); γ is the psychrometric constant (equal to 67 Pa K^{-1}). The terms λE , R_n , G , T of this equation are usually measured in eddy-covariance field campaigns and R_a and R_b are obtained from Eq. 12 and Eq. 13 using other micrometeorological data (e.g. u^*). Saturation water vapour pressure can be obtained by Murray (1967) equation:

$$e_s(T) = 0,611 \cdot \exp[17,269 \cdot (T - 273)/(T - 36)] \quad (16)$$

where T is the air temperature in K. Water vapour pressure can be obtained applying this equation:

$$e = \frac{q \cdot P}{(q - 0.622 \cdot q + 0.622)} \quad (17)$$

where q is the specific humidity and P is the atmospheric pressure.

Since all the other terms of the Eq. 15 are known it is possible to solve this equation in order to obtain R_s , the stomatal resistance (the reciprocal of the stomatal conductance) of water. Since in the sub-stomatal cavity the transport of the molecules is only diffusional, the stomatal conductance of the ozone can be calculated taking into account the following equation:

$$\frac{R_{ST}}{R_s} = \frac{D_{H_2O}}{D_{O_3}} = 1.65 \quad (18)$$

where R_{ST} is the stomatal resistance of ozone in the air, R_s is the stomatal resistance of water in the air, D_{H_2O} is the diffusive coefficient of water in the air and D_{O_3} is the diffusive coefficient of ozone in the air.

Using the Ohm's laws the stomatal fluxes is given by:

$$F_{ST} = \frac{C_c}{R_{ST}} = \frac{R_c}{(R_a + R_b + R_c) R_{ST}} C_m \quad (19)$$

where C_c is the ozone concentration at canopy level.

The stomatal dose is simply given by the integral of F_{ST} over the measuring period.

$$D_{ab} = \int_{t_a}^{t_b} F_{ST}(t) dt \quad (20)$$

3. Applications

Some significant applications at the different scales (leaf, plant and ecosystem level) and their main results will be showed here. A more detailed study on the Mediterranean

vegetation will be presented. In fact, Mediterranean vegetation is often exposed to co-occurring prolonged drought and high ozone concentrations, the effects of drought on stomatal conductance and hence on ozone uptake will be showed.

Karlsson et al. (2000, 2004) developed a model to estimate the stomatal conductance and ozone flux of Norway spruce saplings in an open-top chamber experiment. A Jarvisian model was parameterized against needle conductance measurements, obtained by means of a portable porometer. These measurements were made on 4–6-year-old spruce saplings, grown in open-top chambers, in July–September during three different seasons. The spruce saplings were either maintained well watered or subject to a 7–8 week drought period in July–September each year. The modeled stomatal conductance showed a good agreement with the measured stomatal conductance for the well-watered as well as the drought stress-treated saplings, however significant improvements, applying different VPD functions, were observed. The cumulated ozone uptake showed the occurrence of long-term ‘memory-effects’ from the drought stress treatments on the stomatal conductance. In this paper it was highlighted that these memory-effects should to be considered when simulation models for stomatal conductance are applied to long-lived forest trees under a multiple stress situation. In details, it was observed that the reduction of the total biomass was to 1% per 10 mmol m⁻² of ozone uptake, on a projected needle area basis.

Plejel et al. (2000) reviewed six years of open top chambers experiments on wheat obtaining a specific parameterization of a Jarvisian model. The stomatal model was sensitive to light, temperature and VPD. The relative importance of these climatic factors on stomatal conductance varied considerably between years. VPD was a very important factor limiting daytime ozone uptake in warm and dry summers, while temperature was most important in cool and wet summers. The very strong potential modifying effect by air humidity on ozone effects was described already by McLaughlin & Taylor (1981). Plejel et al. found out that, although ozone concentrations were lower than in south and central Europe, the potential ozone uptake from a given concentration was higher. In fact the long photo-period and the mean values of temperature and humidity lead to relatively great values of stomatal conductance, allowing the vegetation to take up a lot of ozone through the stomatal pathway.

In a two years open-top chambers experiment Gerosa et al. (2009a) realized a reliable parameterization of a stomatal conductance model for four forest species (*Populus nigra* L., *F. excelsior* L., *Fagus sylvatica* L., *Quercus robur* L.) , under the typical meteo-climatic conditions of the Southern Alpine region. The experiment was focused on the determination of a critical level of O₃ uptake for leaf visible injury onset, in different ozone and water availability conditions. It was noticed that seedlings began to suffer the effect of water deficiency as soon as the soil moisture fell just below the field capacity, due both to the soil type and to the incomplete development of their root system. In fact, the correlation between soil water content and stomatal conductance is not linear, because its reduction rate increases as the water shortage persists. This experiment confirmed the influence of an important environmental factor such as soil water availability on plant response to ozone stress.

Sapflow measurements in tree trunks represent a useful link between the leaf and forest canopy level integrating over the entire tree crown and separating tree water flux from other evaporating components in ecosystems (Kostner et al, 2008). However in the same paper the authors highlight some limitations of the sapflow approach: stomatal conductance derived

trunk sapflow may not include higher short term conductances of individual branches in the crown; furthermore, since the foliage transpiration, which starts at sunrise and usually ends at sunset, might be not synchronized with sapflow at breast height, because of the trunk storage capacitance, it was underlined the importance of a proper synchronization between them in order to realize a correct ozone risk assessment. The ozone flux data showed in this paper were in good agreement with the ones presented by Wieser et al. (2000) from gas exchange measurements, proving the goodness of the sapflow approach. It is worth to notice that Kostner et al. (2000) showed the feasibility of the sapflow approach also for measuring NO_x, NH₃ and CO₂ fluxes.

During the exceptionally hot 2003 and the following year, Gerosa et al. (2009b) measured ozone fluxes over a coastal *Q.ilex* forest. From August to October, in 2003 stomatal conductance at canopy level (Gs) was half or less than the following year in the same months. This low values in 2003 can be partially explained, only for August values, with higher temperature and lower water availability. In fact, even after strong rainfalls in September Gs remained low. The authors suggested as a possible explanation that, after a prolonged drought, damages modify the whole plant hydraulic conductivity. As a consequence ozone uptake was nearly double in 2004.

In 2007 a field campaign measuring ozone and energy fluxes over a Mediterranean Maquis ecosystem was run by Gerosa et al. (2009c). Here, additional and different analyses of the data from that field campaign are showed. The field campaign lasted nearly three months from the beginning of May until the end of July. This period corresponds to two different seasonal stages: late spring (until the half of June) and early summer (from the half of June until the end of July). The measuring site was located inside the Castelporziano estate (N 41° 40' 49.3", E 12° 23' 30.6"), 20 km far from the center of Rome (Italy). The studied ecosystem is composed by two different succession stages of the maquis (low and medium maquis). Ninety percent of the ground is covered by 6 main species: *Quercus ilex*, *Arbutus unedo*, *Rosmarinus officinalis*, *Cistus spl*, *Phyllirea latifolia*, *Erica multiflora*. Most of the vegetation height was between 90 cm and 150 cm, with the exception of some higher *Quercus ilex* and *Arbutus unedo* individuals. The eddy covariance technique was used to measure ozone and energy fluxes and a big-leaf approach (Wesely and Hicks, 2000; Gerosa et al., 2003; 2004; 2005) was used to estimate the ozone dose which was taken up by the ecosystem.

The eddy covariance instrumentation (an ultrasonic anemometer (USA-1, Metek, Elmshorn, Germany), a CO₂/H₂O open path fast sensor (LI-7500, LI-COR, Lincoln, Neb., USA) and a fast ozone analyser (COFA, Ecometrics, Italy) were mounted on scaffold and the sampling or measuring point of these instruments was at 3.8 m height. An additional O₃ analyzer (SIR S-5014, DASIBI e, Spain) was used as a reference; the sampling point of the latter instrument was at the same height of the former ones. Additional probes were used to measure net radiation (NR lite, Kipp&Zonen, Holland), photosynthetically active radiation (190SA, LI-COR, Lincoln, Neb., USA), soil water content (C616, Campbell Scientific, Shepshed, United Kingdom), soil heat flux (HFP01SC, Hukseflux, Delft, Holland), leaf temperature (Pt100, DeltaT, United Kingdom), leaf wetness (237, Campbell Scientific, United Kingdom). A temperature and humidity profile (0.1 m, 1 m, 3.8 m) was equipped with three probes (50Y, Campbell Scientific, Shepshed, United Kingdom).

Eddy covariance data were recorded on a computer while for all the other measurements a CR10x datalogger (Campbell Scientific, Shepshed, United Kingdom) was used. Further details about other additional measurements (NO and NO₂ fluxes, which were measured

using the aerodynamic gradient technique) and the data selection and gap-filling can be found in Gerosa et al. (2009c).

The measuring site was characterized by a Mediterranean climate with a strong and prolonged drought, very few rainfall events. A wind breeze regime with wind coming from the inland by night and from the sea during the daylight was observed (Fares et al., 2009). The average temperature in the late spring was 24.0 °C, while in the second part of the field campaign was 28.8 °C. Rainfall was low during the field campaign: 14.5 mm in the first period and nearly null (0.2 mm in the second period) (Fig. 3). Due to the sea breeze regime and the closeness to the sea relative humidity was nearly always above 60% during the day and close to the saturation during the night (Mereu et al., 2009). For this reason, dew formation on leaves happened almost all the nights and, in order to avoid that dew evaporation might be considered as ecosystem transpiration the approach of Gerosa et al. (2009c), considering only data with dry canopy for the resistance analysis and the estimate of the stomatal ozone fluxes, is applied here too.

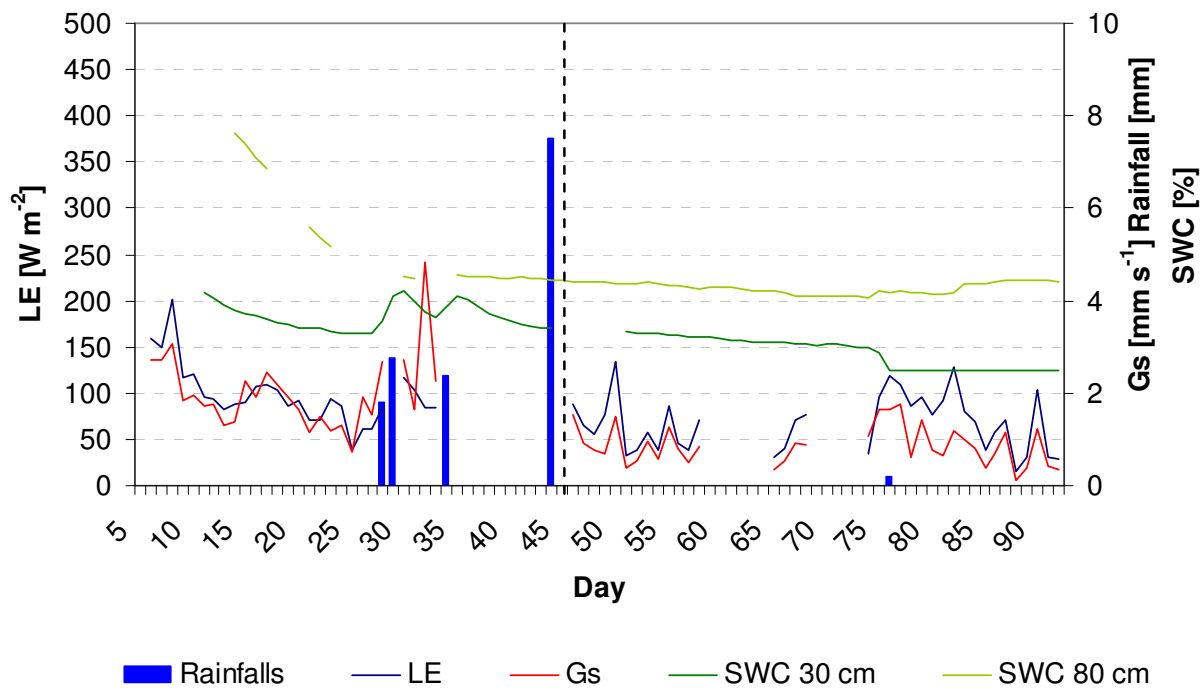


Fig. 3. Daily averages of latent heat flux, stomatal conductance, rain and soil water content (v/v) during the whole measuring period. Soil water was measured at two different levels: 30 cm, 80 cm. Vertical dashed line is the separation between the two periods.

The average ozone concentration on the whole period was 35.9 ppb, lower in the first period (32.6 ppb) and higher in the second (38.6 ppb) (Gerosa et al., 2009c). Ozone concentration showed the typical bell-shaped daily course: from very low concentrations (less than 10 ppb) ozone concentration increased until early in the afternoon reaching its maximum. After that it decreased until sunset, reaching low values for all the night. This behaviour was observed nearly all the days with the exception of few nights when ozone concentration was above 30 ppb (Figure 4).

The total ozone fluxes showed a great daily variability, doubling or halving the daily maximum in one or two days (Figure 4). This great variability is typical of measurements at

ecosystem level where turbulence regulates gas exchange between atmosphere and the ecosystem. The average daily course of the total ozone fluxes course showed two slightly different behaviours in the first and in the second period. In the first one they increased after 9.00 AM and then decreased until midday, after that they increased again until 3.00 PM and then they decreased irregularly until 8.00 PM; the range of the variations was on average between 15 nmol m⁻² s⁻¹ and 20 nmol m⁻² s⁻¹ (Figure 5). In the second part of the field campaign the total ozone fluxes were on average almost constant around 12.5 nmol m⁻² s⁻¹ until 3.00 pm and then decreased until sunset at about 10 nmol m⁻² s⁻¹.

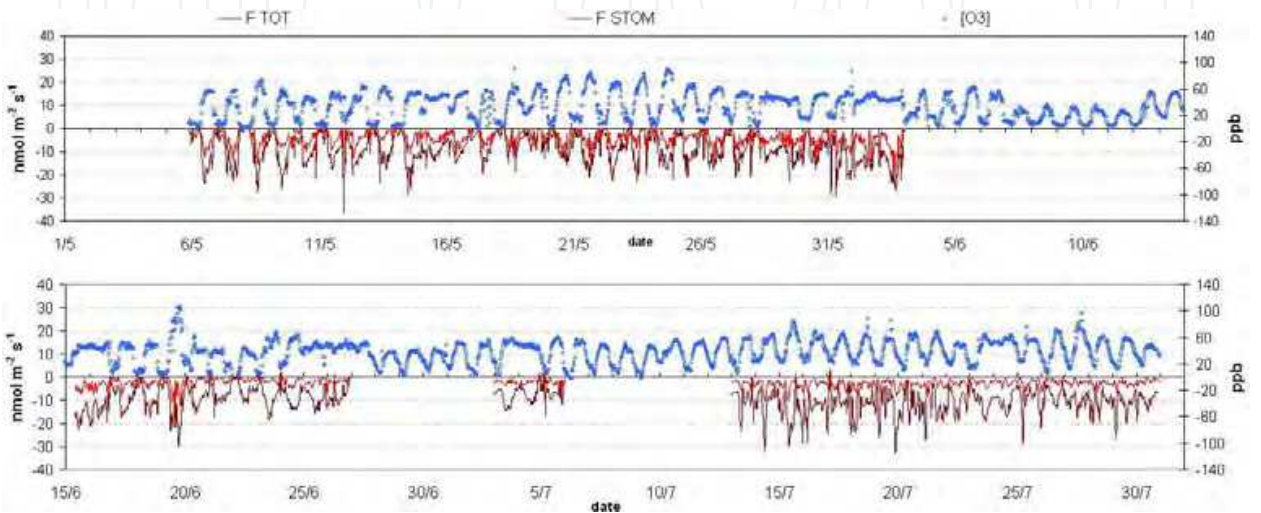


Fig. 4. Ozone concentration and ozone fluxes. The red dark line is the total ozone flux to the ecosystem and the red line is the stomatal flux (left axis), i.e. the amount of ozone taken up by vegetation through stomata. Blue circles represent ozone concentrations (right axis). Modified from Gerosa et al. (2009c).

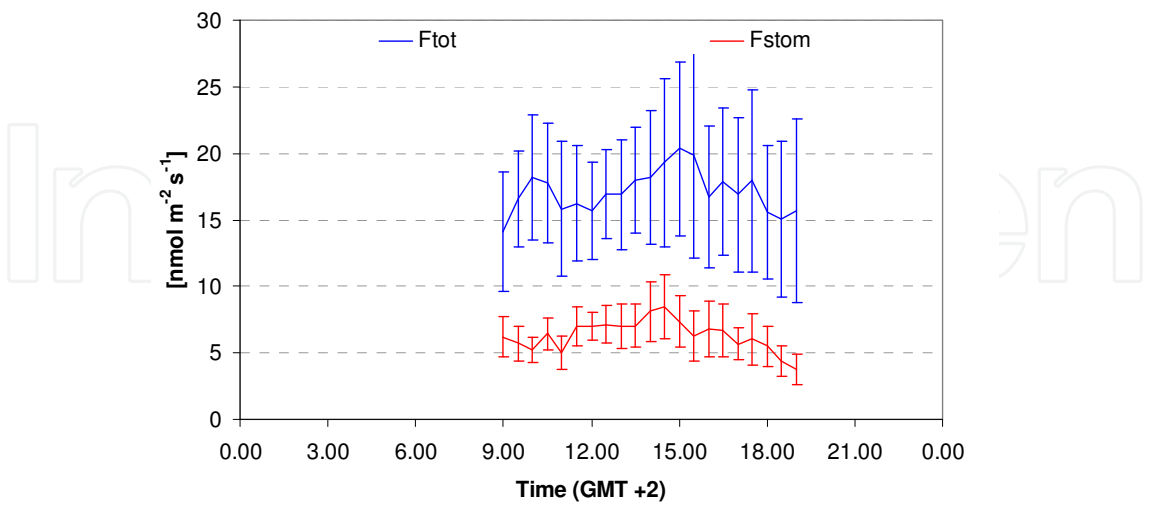


Fig. 5. Mean daily course of the absolute values of total and stomatal ozone fluxes in the first period F_{tot} (blue line) and F_{stom} (red line) are the total and the stomatal ozone fluxes when the canopy were completely dry, i.e. excluding the periods where dew was found on the leaves. Vertical bars are the standard deviations.

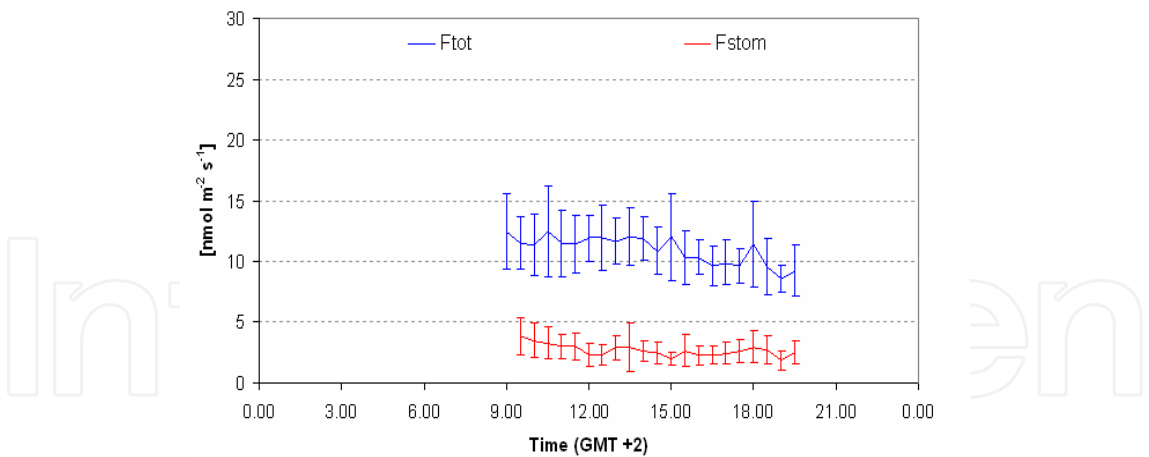


Fig. 6. Mean daily course of the absolute values of total and stomatal ozone fluxes in the second period F_{tot} (blue line) and F_{stom} (red line) are the total and the stomatal ozone fluxes when the canopy were completely dry, i.e. excluding the periods where dew was found on the leaves. Vertical bars are the standard deviations.

The stomatal ozone fluxes showed a daily course similar to the total fluxes of ozone (Figure 5, Figure 6). In the first period the stomatal ozone fluxes were on average around 5 nmol m⁻² s⁻¹ early in the morning, increasing until 8 nmol m⁻² s⁻¹ around 2.00 PM and then decreasing until less than 5 nmol m⁻² s⁻¹ at the sunset. In the second period the stomatal ozone fluxes reached their maximum in the morning (less than 4 nmol m⁻² s⁻¹) and slowly declined for all the day until sunset when they were around 2 nmol m⁻² s⁻¹. Another significant difference about stomatal fluxes between the two periods was the stomatal fraction of the ozone fluxes: as reported by Gerosa et al. (2009c) the stomatal fraction was between 40% and 50% in the first period and between 20% and 30% in the second period. These substantial differences can find an explanation looking at the values of the resistances and conductances calculated by means of the big-leaf model.

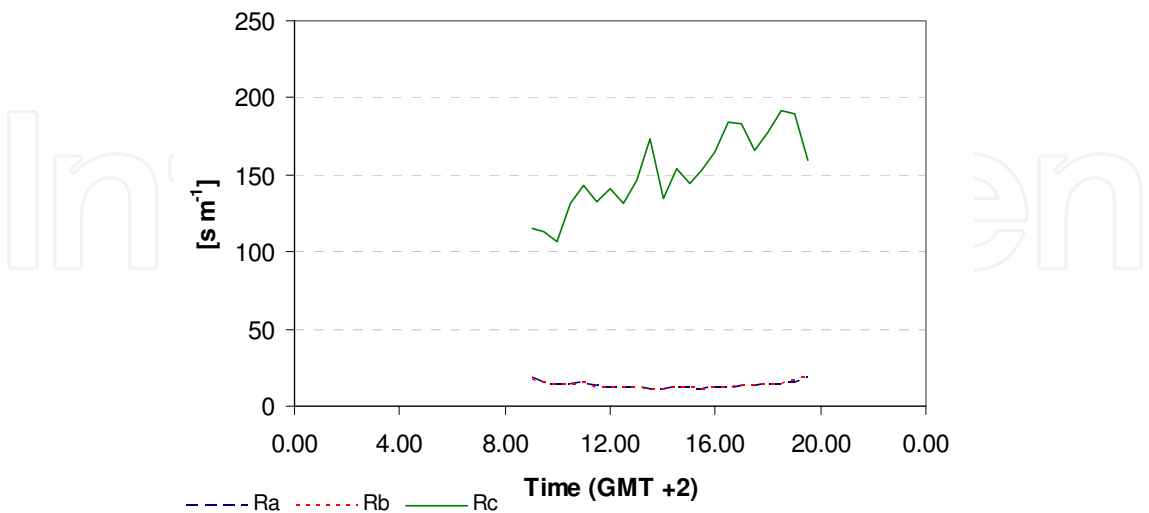


Fig. 7. Mean daily course of the aerodynamic resistance R_a (dashed blue line), of the sublaminal layer resistance R_b (dashed red line) and of the canopy resistance R_c (green line) in the first period.

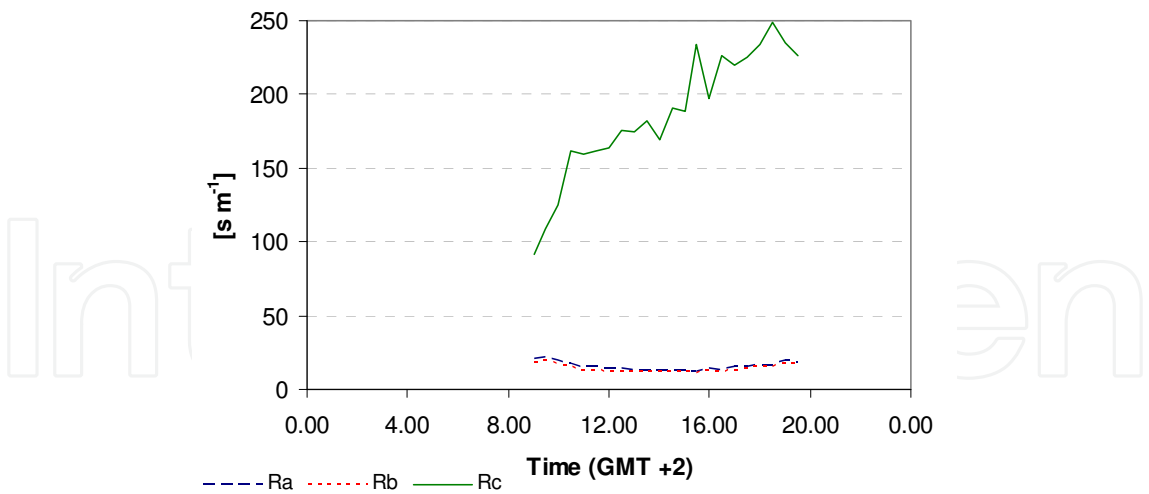


Fig. 8. Mean daily course of the aerodynamic resistance R_a (dashed blue line), of the sublaminar layer resistance R_b (dashed red line) and of the canopy resistance R_c (green line) in the second period.

In both periods R_a and R_b values were significant lower than R_c (Figure 7, Figure 8), showing the major role played by the canopy resistance, and hence by the stomatal and non stomatal pathways, for the ozone deposition. A slightly greater R_c was observed in the second period from late in the morning until the end of the day (Figure 7, Figure 8). Considering now the stomatal and the non stomatal conductance in both periods (Figure 9, Figure 10), a substantial reduction of the stomatal conductance was observed. Its average values in the second period were more than 50% less than in the first period. On the contrary non stomatal conductance showed similar values in the central hours of the day in both period, but was much higher in the morning and in late afternoon in the first half of the measurement campaign.

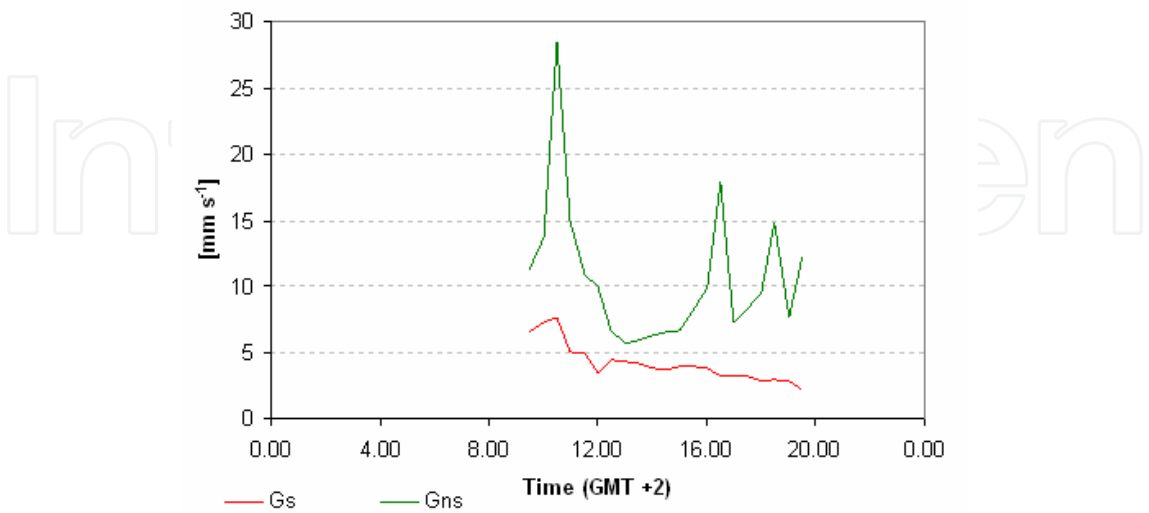


Fig. 9. Mean daily course of the stomatal conductance G_s (red line) and of the non stomatal conductance G_{ns} (green line) in the first period.

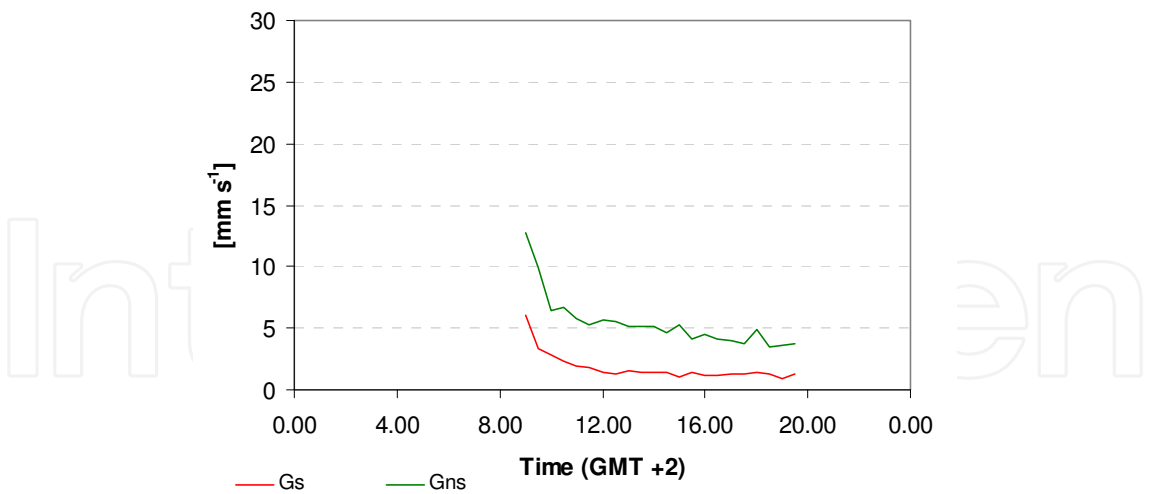


Fig. 10. Mean daily course of the stomatal conductance G_s (red line) and of the non stomatal conductance G_{ns} (green line) in the second period

It is further worth to notice the good agreement between the daily averages of stomatal conductance, soil water content (at two depth) and latent heat fluxes, which is proportional to (evapo)transpiration. Soil water shortage affected stomatal conductance, which in turn affected transpiration: in fact, in the first half of the field campaign soil water content decrease at both depth was in good agreement with the decrease of both stomatal conductance and latent heat fluxes, while in the second half they were all nearly constant (Figure 3).

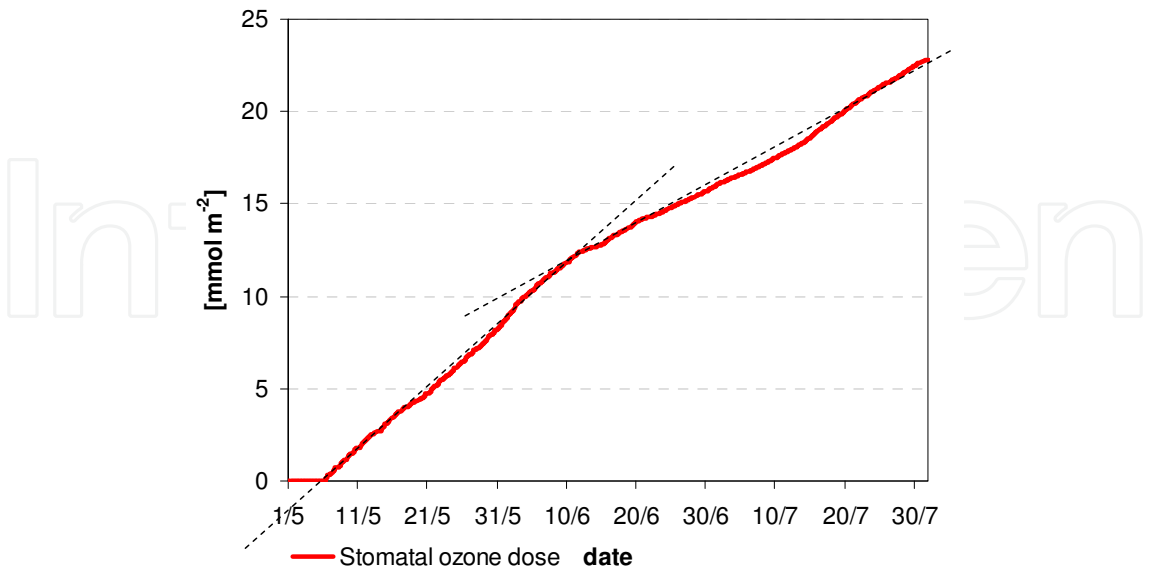


Fig. 11. Evolution of ozone stomatal dose during the measuring period.

The stomatal fluxes, after gap-filling procedure, were all summed up to calculate the ozone dose taken up by the ecosystem, the ozone dose resulted 22.8 mmol m⁻². Considering the accumulation of the ozone dose during the field campaign (Figure 11) two different growth rates of the cumulated dose were observed, mirroring the different behaviours of the stomatal conductance and thus of the fluxes in the two periods of the field campaign.

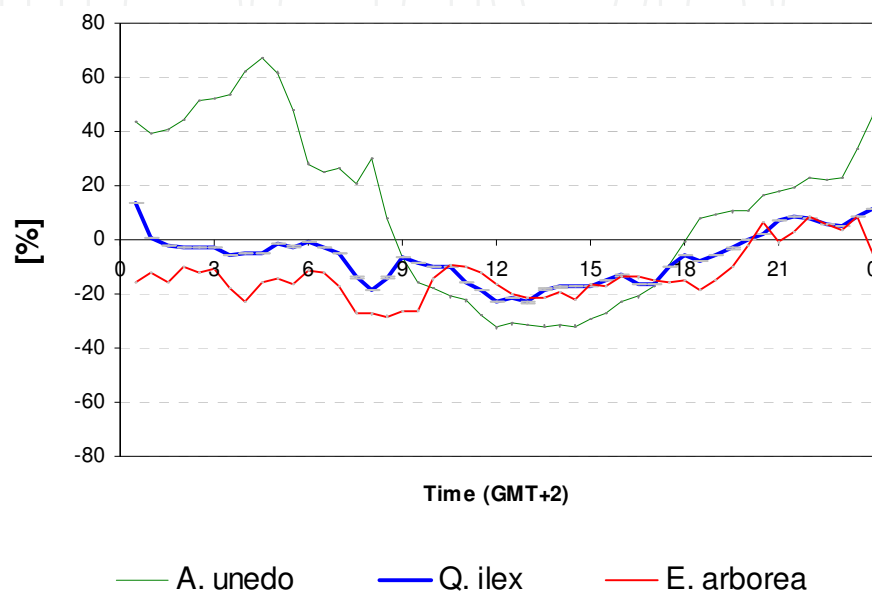


Fig. 12. Percentage difference between the two periods for the transpiration of the three species. Negative values correspond to a decrease in the second period and positive values correspond to an increase.

During this field campaign, Mereu et al. (2009) performed sap flow measurement over three species (*A. unedo*, *Q. ilex*, *E. arborea*). Unfortunately these three species represented only 38% of the total cover and hence it was not possible to test any up-scaling procedure, however some interesting features were observed. First of all it was possible to realize that the stomatal conductance peak, observed early in the morning by means of eddy covariance measurements and the following analyses, was probably due to the evaporation of dew from leaves (Gerosa et al., 2009c). In fact none of the three studied species showed any stomatal conductance peak early in the morning and the other three main species are known to not show any behaviour like this (Gerosa et al., 2009c). For this reason only data when canopy was dry were used to estimate canopy stomatal conductance and fluxes. Another important feature that was highlighted by sap flow measurements was the differences among the studied species. For instance, in both periods *Q.ilex* transpiration (which is directly proportional to stomatal conductance) increased earlier than the two other species, but, in the central hours of the day, it was from 10% to 25% less than *E. arborea* transpiration and from 40% to 50% less than *A. unedo*. Furthermore, if we consider the percentage

variation of the transpiration of each species some interesting features can be observed. All the three species showed a sensible decrease in the daylight hours (from 15% to 30%) while after the sunset for all the three species an increase of the transpiration (more remarkable for *A. unedo*) was observed during the second period. From noon to the dawn the behaviour of the three species was different in the second period: *A. unedo* increased the night time transpiration of 40% on average, *Q. ilex* showed a slight reduction (about 5%) and *E. arborea* a more marked reduction (15-20%).

As already observed by Gerosa et al. (2009c), this analysis remarks the importance of considering plant physiology when studying multi-species ecosystem, in fact the different behaviours of these three species led to different ozone uptake by them and thus to different level of ozone risk for each species.

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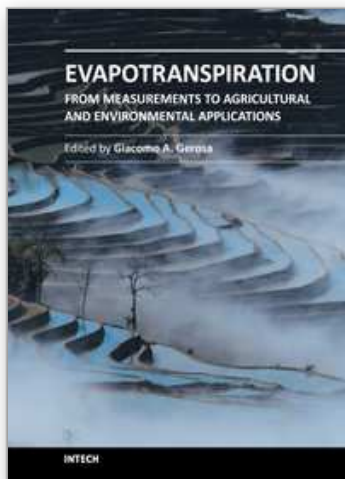
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Evapotranspiration - From Measurements to Agricultural and Environmental Applications

Edited by Dr. Giacomo Gerosa

ISBN 978-953-307-512-9

Hard cover, 410 pages

Publisher InTech

Published online 09, November, 2011

Published in print edition November, 2011

This book represents an overview of the direct measurement techniques of evapotranspiration with related applications to the water use optimization in the agricultural practice and to the ecosystems study. Different measuring techniques at leaf level (porometry), plant-level (sap-flow, lysimetry) and agro-ecosystem level (Surface Renewal, Eddy Covariance, Multi layer BREB), are presented with detailed explanations and examples. For the optimization of the water use in agriculture, detailed measurements on transpiration demands of crops and different cultivars, as well as results of different irrigation schemes and techniques (i.e. subsurface drip) in semi-arid areas for open-field, greenhouse and potted grown plants are presented. Aspects on ET of crops in saline environments, effects of ET on groundwater quality in xeric environments as well as the application of ET to climatic classification are also depicted. The book provides an excellent overview for both, researchers and student,s who intend to address these issues.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Giacomo Gerosa, Angelo Finco, Simone Mereu, Antonio Ballarin Denti and Riccardo Marzuoli (2011). (Evapo)Transpiration Measurements Over Vegetated Surfaces as a Key Tool to Assess the Potential Damages of Air Gaseous Pollutant for Plants, Evapotranspiration - From Measurements to Agricultural and Environmental Applications, Dr. Giacomo Gerosa (Ed.), ISBN: 978-953-307-512-9, InTech, Available from: <http://www.intechopen.com/books/evapotranspiration-from-measurements-to-agricultural-and-environmental-applications/-evapo-transpiration-measurements-over-vegetated-surfaces-as-a-key-tool-to-assess-the-potential-dama>

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