Improvement of drought tolerance in maize (*Zea mays L.*) by selected rhizospheric microorganisms

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Abstract: *Several beneficial rhizospheric microorganisms, such as arbuscular mycorrhizal fungi (AMF) and Plant Growth Promoting Rhizobacteria (PGPR), can improve the tolerance of plants to water deficiency and other environmental stresses. In this work the addition to soil of selected rhizospheric microbial consortia was tested in order to increase tolerance to drought stress of maize crops. One consortium was a mixture of AMF fungi and PGPR isolated from an arid soil of Senegal and selected under osmotic stress conditions (M1); the other consortium was an unspecific commercial product (M2). The soil microbial populations and the root infection by AMF were affected by the inoculation with both consortia. Plant gas exchange parameters were positively affected by inoculation, with different responses depending on the consortium. A higher tolerance of plants to water deficiency stress was reached with consortium M1. Improvement of the mineral nutrients content of leaves and of the biomass yield was also recorded. It is concluded that the use of microbial inocula of rhizospheric microorganisms specifically selected for drought tolerance can be suitable for agronomical applications, aimed at improving the crop growth and yielding performances under low water availability.*

Keywords: *maize; drought; climate change, adaptation, soil microorganisms.*

Riassunto: *Diversi microrganismi rizosferici, come ad esempio i funghi micorrizici arbuscolari (AMF) e i rizobatteri promotori della crescita delle pianta (PGPR), possono migliorare la resistenza delle piante alla carenza di acqua e ad altri stress ambientali. In questo lavoro è stato testato l'effetto dell'inoculo nel terreno di consorzi microbici rizosferici selezionati al fine di aumentare la tolleranza allo stress da siccità per la coltura del mais. Un consorzio era costituito da un insieme di AMF e PGPR isolato da un suolo arido del Senegal e selezionato in condizioni di stress osmotico (M1), il secondo era costituito da un prodotto commerciale non specifico (M2). L'inoculo delle popolazioni microbiche nel suolo e l'infezione della radice per mezzo di AMF è stato effettuato con entrambi i consorzi. Gli scambi gassosi delle piante sono stati positivamente influenzati dall'inoculo, ottenendo risposte diverse a seconda del consorzio. Una maggiore tolleranza delle piante allo stress idrico è stata ottenuta con il consorzio M1. E' stato anche registrato un miglioramento del tenore di nutrienti minerali delle foglie e della produzione di biomassa. Si è concluso che l'uso di inoculi microbici composti da microrganismi rizosferici specificatamente selezionati per la tolleranza alla siccità può essere adatto per applicazioni agronomiche, allo scopo di migliorare la crescita delle colture e ottenere migliori prestazioni produttive in condizioni di scarsa disponibilità idrica.*

Parole chiave: *mais; siccità; cambiamento climatico; adattamento; microrganismi del suolo.*

Abbreviations

A: CO_2 uptake (μmol CO_2 m^{-2} s^{-1})
AMF: Arbuscular Mycorrhizal Fungi
Ci: internal CO_2 (ppm)
C.I.: Chlorophyll Index
CFU: Colony Forming Units
DAS: Days After Sowing
E: leaf transpiration rate (mmol H_2O m^{-2} s^{-1})
g_s: stomatal conductance (mol H_2O m^{-2} s^{-1})
MHB: Mycorrhizal Helper Bacteria
PGPR: Plant Growth Promoting Rhizobacteria
WC: Water Content
WUE: water use efficiency (mmol CO_2 · mmol H_2O^{-1})

1. INTRODUCTION

In order to face the negative effects determined by the current climatic anomalies and extreme events numerous studies have been carried out to evaluate and propose mitigation measures and to develop adaptation techniques in agriculture (Chatzidaki and Ventura, 2010).

In fact, it is well known that in temperate countries climate change is the origin of increased frequency of climatic anomalies and unusual extreme events, such as drought, long dry seasons, heatwaves and short and intense rainfall.

In particular, limited water availability for agriculture is a permanent problem in hot-arid countries, but also a rising environmental and social problem in temperate countries, where it could severely limit crops yield due to competition between agricultural

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and other uses of water resources (Villani *et al.*, 2011). At regional level, studies have been conducted to formulate future scenarios of climate change in relation to the availability of water. In northern Italy, in particular in the Po Valley, future scenarios predict a reduction of water availability (Bisaglia *et al.*, 2011; Gallo *et al.*, 2012). In order to adapt to these future scenarios many studies are aimed to improve drought tolerance of plants either through genetic approaches, as well as by biotechnological and agronomical practices (Fleury *et al.*, 2010). Drought stress drastically reduces plant growth and crop yielding, mainly by reducing the plant photosynthetic activity due to stomatal closure, or by structural and functional changes in the photosynthetic apparatus (Cornic and Masacci, 1996; Muller and Whitsitt 1996; Harley and Smith 1983; Fitter, 1988). Among agronomical practices aiming at improving the crops drought tolerance, the inoculation with rhizospheric beneficial microorganisms has been proposed, in particular with arbuscular mycorrhizal fungi (AMF) (Augé, 2001). Plants absorb water from the layer of soil which is in the near proximity to the roots, as the water depletion zone spans few millimetres. The increased amount of water taken up by mycorrhized plants could be partially ascribed to the larger volume of soil explored by mycelial hyphae with respect to the volume of soil directly explored by plant roots, which results in increased soil/hyphae interface (Smith and Read, 2008). Plant-soil water relations and gas exchanges are affected by AMF symbiosis also through physiological mechanisms: enhanced nutrient uptake, increased stomatal conductance, lowered leaf osmotic potential, influence on cytokinin homeostasis (Augé, 2000; Subramanian and Charest, 1995, 1999). However AM fungi strongly interact with the other soil microorganisms. The interaction is acting either ways: AM inoculation improves the establishment of inoculated and indigenous rhizobacteria, which may act as mycorrhizal helper bacteria (MHB) thus increasing root mycorrhization efficiency (Frey-Klett *et al.*, 2007). Studies on multi-microbial interactions, including those between AM fungi and different PGPR species are pointing out that microorganisms act synergistically when inoculated simultaneously (Barea *et al.*, 2002; Gamalero *et al.*, 2004). Several PGPR have been recognised as capable of alleviating the consequences of drought stress in plants by different mechanisms, such as production of phytormones that enhance root growth and formation of lateral roots and, consequently, nutrients and water uptake, reduction of ethylene concentration inside the plant by production of ACC-deaminase (Bardi and Malusa' 2012). Both AM fungi and PGPR can improve the soil structure and water retention properties by forming and stabilizing soil aggregates through deposition of organic compounds such as glycoproteins or exopolysaccharides (Bardi and Malusà, 2012).

Several studies have been reported in which selected AM fungi and rhizospheric bacteria improved adaptation to water deficiency conditions as well as to saline soils (Tao and Zhiwei, 2005; Mayak *et al.*, 2004; Egamberdieva and Kucharova, 2009). However, plant inoculation with beneficial microorganisms has limited effects unless the environment supports growth and survival of the introduced micro-organisms; the survival of the inoculated microorganisms largely depends on the capacity of competing with the better adapted native microflora (Rekha *et al.*, 2007). Thus, to get significant benefits from inoculation with beneficial microorganisms, the selection of most effective root-colonising AMF and bacterial strains, specifically selected for the tolerance to an environmental stress factor, is a prerequisite. Trials applying selected strains have mainly be limited to the use of either AMF or PGPR alone, nevertheless consortia of these micro-organisms are probably more adapted to survive and predominate when introduced into a natural environment (Barea *et al.*, 2002).

A rhizosferic microbial consortium was selected from an arid soil of the sahelian region of Louga (Senegal) and tested in maize crops, showing a better biomass yield at different water regimes and allowing the production of ears while drought stress prevented it in plants without inoculum (Bardi and Malusa', 2012). The aim of the present work was to assess the physiological response to water deficiency of maize plants inoculated with a consortium of rhizospheric beneficial microorganisms (AMF and PGPR) specifically selected from an arid environment: the consortium was made of a pool of AMF and PGPR strains isolated from sandy, saline soils of the sahelian region of Louga (Senegal) and selected under osmotic pressure. A comparison to plants without inoculum and to plants inoculated with a commercial, non drought-specifically selected microbial consortium was carried out.

2. MATERIALS AND METHODS

2.1. Rhizospheric microbial consortia

Drought-tolerant consortium (M1)

A consortium of drought-tolerant rhizospheric microorganisms (M1) was formed as a pool of AMF and bacterial strains selected from the rhizospheric soil of tomato plants collected in the Region of Louga in Senegal, characterized by sahelian climate and sandy and saline soils. AMF were selected and propagated according to Luster and Finlay (2006) with *Sorghum bicolor* as host plant. Two bacterial strains, named PS30 and BA30, were selected under osmotic selective pressure. PS30 was isolated on King Agar B (Fluka) for *Pseudomonas spp.* strains; BA30 was isolated on TSA (Oxoid) for *Bacillus spp.* strains. Both growth media were added with $30\ g \cdot l^{-1}$ NaCl to select strains tolerant to osmotic stress. Both strains were identified by 16S rDNA sequence similarity. Amplification and sequencing reactions were performed according to Lane (1991). The primers used were 27F AGAGTTTGATCATGGCTCAG and 704R TCTACGCATTTCACCGCTAC. A Beckman CEQ8000 automated DNA sequencer was used according to the manufacturer's instructions. The most similar sequences were found using BLAST search from the NCBI's databases (http://www.ncbi.nlm.nih.gov/BLAST; Nucleotide collection (nr/nt)); BA30 showed 100% sequence similarity to *Bacillus subtilis*; PS30 showed 98% sequence similarity to *Halomonas sp.*

Commercial consortium (M2)
A commercial unspecific consortium (M2) (Micosat F, CCS Aosta s.r.l., Italy) was also used in the trial. It was composed of three AMF species (*Glomus caledonium* GM24, *Glomus intraradices* GG31, *Glomus coronatum* GU53, in form of spores, hyphae and root fragments), and three PGPR species (*Pseudomonas fluorescens* PA28, *Pseudomonas borealis* PA29, *Bacillus subtilis* BA41) with a total concentration of 10^6 cells $\cdot\ g^{-1}$.

2.2. Experimental set up

The trial was carried out in mesocosms established in a greenhouse. The mesocosms were formed of plastic bags of 1 mc of volume of soil each. They were placed in a trench and the soil used to fill them was previously uniformly mixed. Soil texture and composition was: sand 76.5%, lime 20%, clay 3.5%; pH 7.5; salinity 3 Ec; P 12.9 mg $\cdot$ 100 g^{-1}; K 7.71 mg $\cdot$100 g^{-1}; Mg 16.1 mg $\cdot$100 g^{-1}; total N 0.13%; total C 0.35% equivalent to 0.6% organic matter.
Six maize plants, cv. NK Famoso Syngenta (FAO class 500-600), were grown in each mesocosm. Before sowing, the soil was fertilized with dry manure, at a dose equivalent to 10 t $\cdot$ ha^{-1}.
Plants were treated with the two different inocula previously described; control plants were not inoculated (M0). The inocula were applied at sowing to the soil near the seed (1 g $\cdot$ $plant^{-1}$). Sowing was carried out on June 5th and plants were watered, from sowing until bloom, at two weeks intervals with an adequate water volume to restore field capacity. In correspondence of the irrigation episode in July, six weeks after sowing, samples were collected and analysis were carried out on plants under water deficiency (14 days after irrigation) and in the recovery phase (1 day after irrigation) to determine: chlorophyll index, stomatal conductance, transpiration rate, CO_2 uptake, internal CO_2, water use efficiency, leaf water content. All the assays were executed in triplicate. Soil moisture tension was monitored throughout the experiment in M0 and M1 mesocosms by Watermark Soil Moisture Sensor and WatchDog™ Data loggers (Spectrum Technologies, Inc.).

2.3. Determination of the chlorophyll index

The chlorophyll index (C.I.) was measured with the SPAD 502 chlorophyll meter, which determines the relative amount of chlorophyll in the leaf by measuring the absorbance in the red and near-infrared regions. The instruments calculate an unitless value, which is proportional to the amount of chlorophyll present in the leaf itself. Chlorophyll meter readings were taken from each plant on 30 randomly selected points from the uppermost leaf of three plants (10 measures per plant) present in each mesocosm.

2.4. Determination of gas exchange parameters

Leaf transpiration rate (E), stomatal conductance (g_s), CO_2 uptake (A) and internal CO_2 (Ci) were recorded using an infra-red gas analyzer ADC-LCPro+ system (The Analytical Development Company Ltd, Hoddesdon, Herts, UK). The measurements were done on three leaves per plant inserted in the central region of the shoot (nodes 4 to 6 from the shoot base). The results were expressed respectively as follows: E mmol $H_2O\ m^{-2}\ s^{-1}$, g_s mol $H_2O\ m^{-2}\ s^{-1}$, A µmol $CO_2\ m^{-2}\ s^{-1}$, Ci ppm; water use efficiency (WUE) was calculated as $A \cdot E^{-1}$ and expressed as mmol $CO_2 \cdot$ mmol H_2O^{-1}.

2.5. Morphometric analysis of plants

The biomass yield of each plant was determined by measuring the dry weight of the shoots and roots separately at the end of the vegetative cycle in September. Plant tissues were dried in oven at 70°C and the weight of each single plant was measured separately.
Dry weight and water content of a single leaf from

each plant was also measured at the same time of the determinations of the gas exchange parameters and chlorophyll index. Water content (WC) was calculated as the difference between the fresh weight measured at the time of sampling and the dry weight and reported as % of fresh weight ([(fresh wt. – dry wt.)/fresh wt.] x 100).

2.6. Determination of leaves mineral content

Plant material for the analysis of mineral composition was dried at 60 ºC for 48 h, and ground to pass a 1 mm-sieve. Total nitrogen was determined using the standard Kjeldahl method after wet mineralization (Ostrowska *et al.* 1991).

The grounded plant material was mineralized in teflon-coated containers in a microwave oven in a 5:1 mixture of HNO_3 and H_2O_2, under controlled temperature and pressure, to determine the macro and microelements content by ICP spectrometry (Cygański, 1997).

2.7. Determination of rhizospheric cultivable microbial populations and of roots AMF colonization

The soil collected with the root samples was used to determine the microbial population in rhizospheric soil. The soil was suspended into sterile saline solution (NaCl 0.9%) and then the suspension, opportunely diluted, was used to inoculate Petrifilm™ AC plates to determine aerobic and anaerobic microbial populations and Petrifilm™ YM plates to determine yeasts and fungi populations. The plates were incubated at 25°C for four days; for the determination of anaerobic bacterial populations the plates were incubated in Anaerojar (Oxoid) in anerobiosis generated by the kit Anaerogen (Oxoid). Colony forming units (CFU) were counted and the concentration of each microbial population was expressed as CFU · g^{-1} soil (dry weight).

Root samples were collected twice during the growth cycle: the first sampling was carried out 40 days after sowing (DAS) and the second at 100 DAS, at the end of the growth period. Roots from each plant were carefully washed and stained (Phillips and Hayman, 1970). Mycorrhizal colonization percentage was determined using a grind-line intersect method (Giovannetti and Mosse, 1980).

2.8. Statistical analyses

Data were analyzed by ANOVA with SPSS software; means were compared by LSD multivariate post hoc at P ≤ 0.05.

3. RESULTS

3.1. Chlorophyll index

No significant differences were observed for the chlorophyll index (C.I.) among M0, M1 and M2 plants; however, during recovery phase after the irrigation, an increase of the index was recorded for all treatments, but markedly in M2 plants (C.I. = 61) (Fig. 1).

3.2. Gas exchange parameters

The stomatal conductance was significantly higher in inoculated plants (M1 and M2), reaching values more than double with respect to uninoculated plants; moreover, during recovery phase after the irrigation a strong increase of the stomatal conductance was observed, particularly in M1 plants

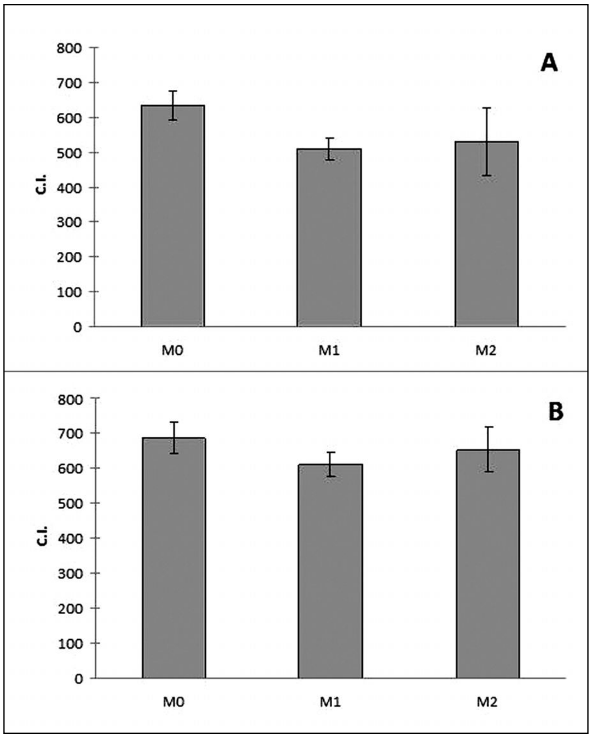

Fig. 1 - Chlorophyll Index of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. A: water deficiency phase (14 days after irrigation); B: recovery phase (1 day after irrigation).

Fig. 1 - Indice clorofilliano delle piante di mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. A: fase di carenza idrica (14 giorni dopo l'irrigazione); B: fase di recupero (1 giorno dopo l'irrigazione).

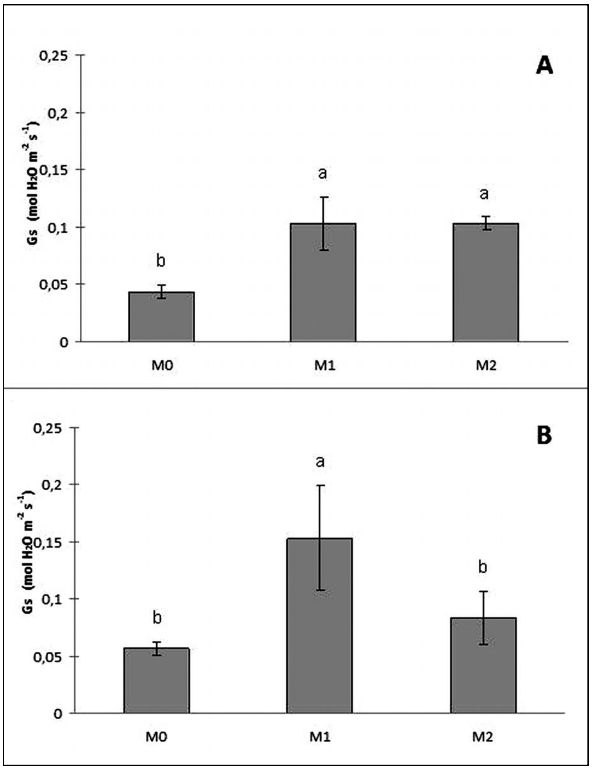

Fig. 2 - Leaf stomatal conductance (g_s) of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. A: water deficiency phase (14 days after irrigation); B: recovery phase (1 day after irrigation). Bars with different letters are significantly different at P≤0.05 according to LSD test.

Fig. 2 - Conduttanza stomatica fogliare (gs) delle piante di mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. A: fase di carenza idrica (14 giorni dopo l'irrigazione); B: fase di recupero (1 giorno dopo l'irrigazione). Barre con lettere diverse sono significativamente differenti per P ≤ 0,05 secondo il test LSD.

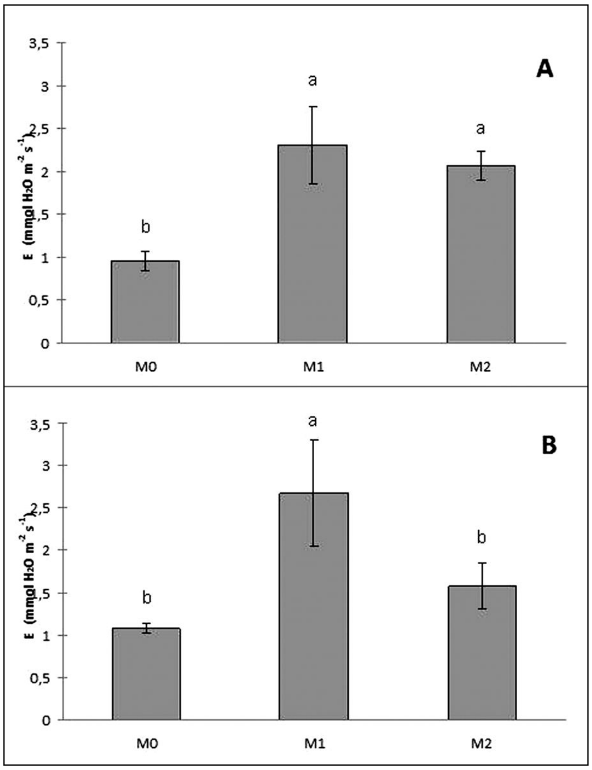

Fig. 3 - Leaf transpiration rate (E) of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. A: water deficiency phase (14 days after irrigation); B: recovery phase (1 day after irrigation). Bars with different letters are significantly different at P≤0.05 according to LSD test.

Fig. 3 - Tasso di traspirazione fogliare (E) delle piante di mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. A: fase di carenza idrica (14 giorni dopo l'irrigazione); B: fase di recupero (1 giorno dopo l'irrigazione). Barre con lettere diverse sono significativamente differenti per P ≤ 0,05 secondo il test LSD.

(Fig. 2). The lowest value was detected for M0 plants before irrigation (0.043 ± 0.006 mol H_2O m^{-2} s^{-1}), and the highest for M1 plants after irrigation (0.153 ± 0.046 mol H_2O m^{-2} s^{-1}).

The transpiration rate was higher in inoculated plants in comparison to untreated plants both during the water stress and after irrigation (Fig. 3). Furthermore, the plants inoculated with the specifically selected consortium M1 showed the highest values, significantly different also from the plants inoculated with the commercial consortium M2 in the recovery phase (Fig. 3). The transpiration rate ranged from 2.673 ± 0.630 mmol H_2O m^{-2} s^{-1} in M1 plants after irrigation to 0.957 ± 0.115 mmol H_2O m^{-2} s^{-1} in M0 plants during the water stress period. CO_2 uptake was significantly higher in M1 and M2 plants than in M0 plants under water deficiency; after irrigation, CO_2 uptake increased in M1 plants, resulting significantly higher than in both M0 and M2 plants (Fig. 4). The lowest value was detected for M0 plants under water deficiency (8.370 ± 2.176 mmol µmol CO_2 m^{-2} s^{-1}), and the highest in M1 plants in recovery phase (24.603 ± 7.210 mmol CO_2 m^{-2} s^{-1}).

No statistically significant differences were observed for the water use efficiency parameter, neither under

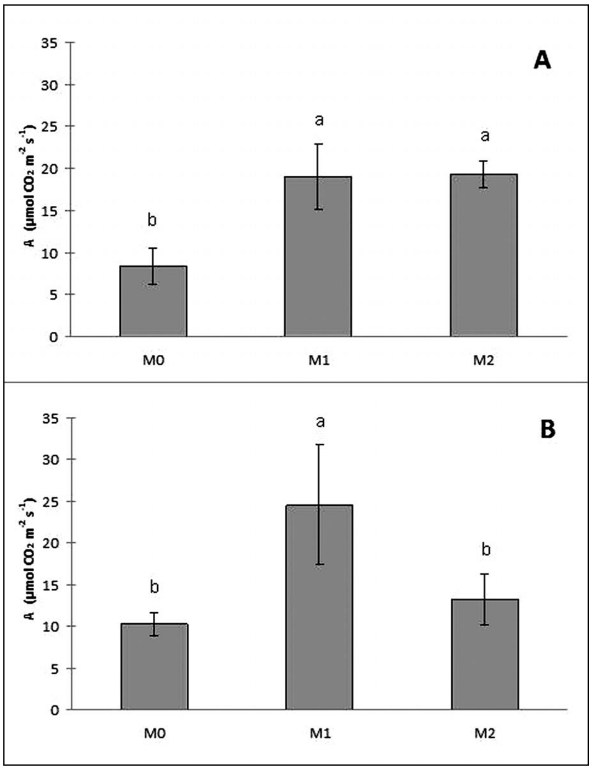

Fig. 4 - Leaf CO_2 uptake (A) of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. A: water deficiency phase (14 days after irrigation); B: recovery phase (1 day after irrigation). Bars with different letters are significantly different at P≤0.05 according to LSD test.

Fig. 4 - Assimilazione fogliare di CO_2 (A) delle piante de mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. A: fase di carenza idrica (14 giorni dopo l'irrigazione); B: fase di recupero (1 giorno dopo l'irrigazione). Barre con lettere diverse sono significativamente differenti per P ≤ 0,05 secondo il test LSD.

water deficiency nor in the recovery phase. However, during the recovery period a contrasting behavior was observed between M0 and M1 plants, from one side, and M2 plants from the other: the former treatments showed an increase in WUE in comparison to the value measured under water deficiency, while M2 plants showed a small decrease (Fig. 5).

Substomatal CO_2 concentration in inoculated plants was lower (one third) than in uninoculated controls upon water stress (Fig. 6, A); after soil irrigation, substomatal CO_2 concentration did not change in M0 plants, whereas it was 2.5 times higher in M1 ones, reaching levels of control plants, and 5 times higher in M2 ones (Fig. 6, B).

3.3. Morphometric analysis of plants

The average total biomass yielded by M1 treated plants was about 30% and 10% higher than M2 and M0 plants, respectively (Fig. 7). However, the high variability of the data, particularly those from M2 plants, resulted in the absence of statistically significant differences between the treatments. Root biomass was lower in M1 and M2 plants than in M0 plants (Fig. 7). Also in this case, the data for M1 plants showed the lowest variability.

The water content of leaves sampled in July under water deficiency and during recovery after irrigation did not show significant differences, but it was slightly higher in M2 plants after irrigation (Fig. 8).

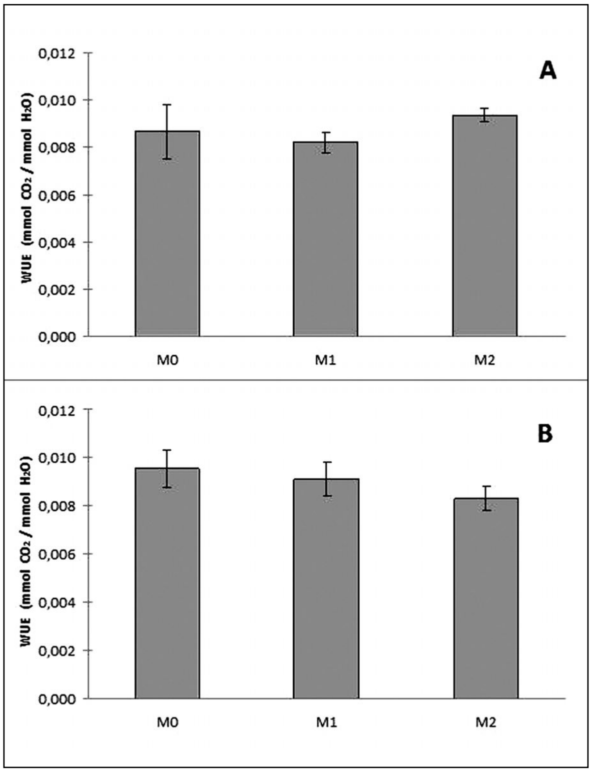

Fig. 5 - Water use efficiency (WUE) of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. A: water deficiency phase (14 days after irrigation); B: recovery phase (1 day after irrigation).

Fig. 5 - Efficienza d'uso dell'acqua (WUE) delle piante di mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. A: fase di carenza idrica (14 giorni dopo l'irrigazione); B: fase di recupero (1 giorno dopo l'irrigazione).

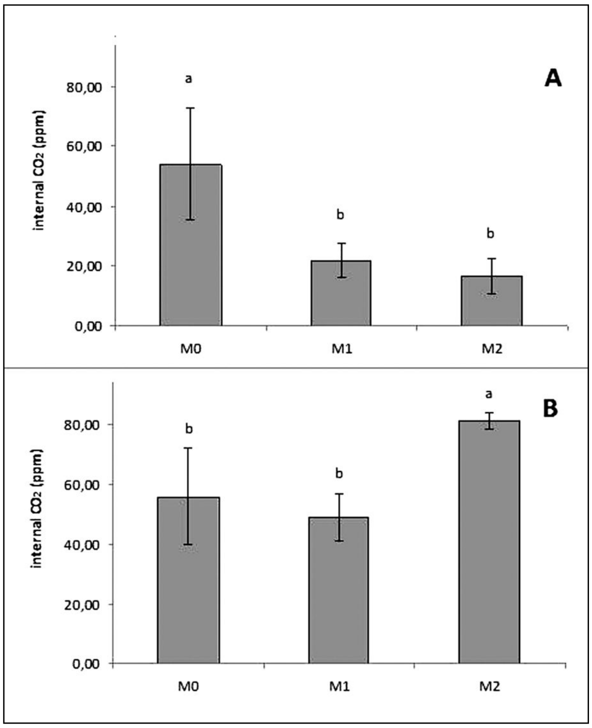

Fig. 6 - Internal CO_2 (Ci) of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. A: water deficiency phase (14 days after irrigation); B: recovery phase (1 day after irrigation). Bars with different letters are significantly different at $P \leq 0.05$ according to LSD test.

Fig. 6 - CO_2 Interna delle piante di mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. A: fase di carenza idrica (14 giorni dopo l'irrigazione); B: fase di recupero (1 giorno dopo l'irrigazione). Barre con lettere diverse sono significativamente differenti per P ≤ 0,05 secondo il test LSD.

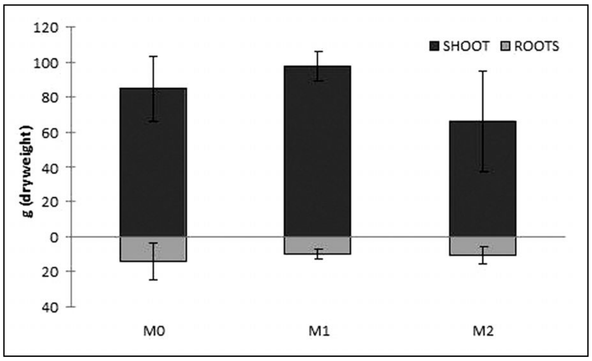

Fig. 7 - Shoots and roots biomass of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. (The values for roots below the X axis are positive).

Fig. 7 - Produzione di biomassa di radici e parti aeree delle piante di mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale (I valori riferiti alle radici al di sotto dell'asse X sono positivi).

3.4. Leaves mineral content

Even though differences between the treatments were not statistically significant, in general a higher content of mineral nutrients was found in M1 and M2 plants than in M0 plants, with the exception of potassium (Tab. 1).

3.5. Rhizospheric cultivable microbial populations and roots colonisation with AMF

Plants inoculated with both consortia showed larger populations of aerobic bacteria in their rhizospheric soil in comparison to not inoculated plants (Tab. 2). Anaerobic bacteria were found to a higher amount associated with untreated and M1 roots with respects to M2 plants. Rhizospheric soil contained a significantly higher number of yeasts and fungi populations in M0 plants, in comparison to inoculated plants. M1 treated roots showed the lowest number of populations of these microorganisms (Tab. 2).

The rate of roots colonized by AM fungi was very high in both inoculated and uninoculated plants in correspondence of both sampling dates, carried out 40 Days After Sowing (DAS) and at the end of the growth period (100 DAS). However, the rate varied when comparing the data from the two sampling dates: it decreased with the time in M0 plants, while it increased in M1 and M2 plants (Fig. 9), reaching the values of 36,4%, 49,9% and 32,6%, respectively.

3.5. Soil moisture tension

Soil moisture tension showed a drop in correspondence of each irrigation followed by a rising trend; after the last irrigation the soil moisture tension rose more slowly in M1 than in M0 mesocosms (Fig. 10).

4. DISCUSSION

The application of microbial consortia to maize plants experiencing a water deficiency period dramatically affected the physiology of the plants and their rhizospheric microbial population. However, the kind of strains composing the consortium, specifically selected from an arid soil or not selected for this trait, was a major factor influencing the plant response.

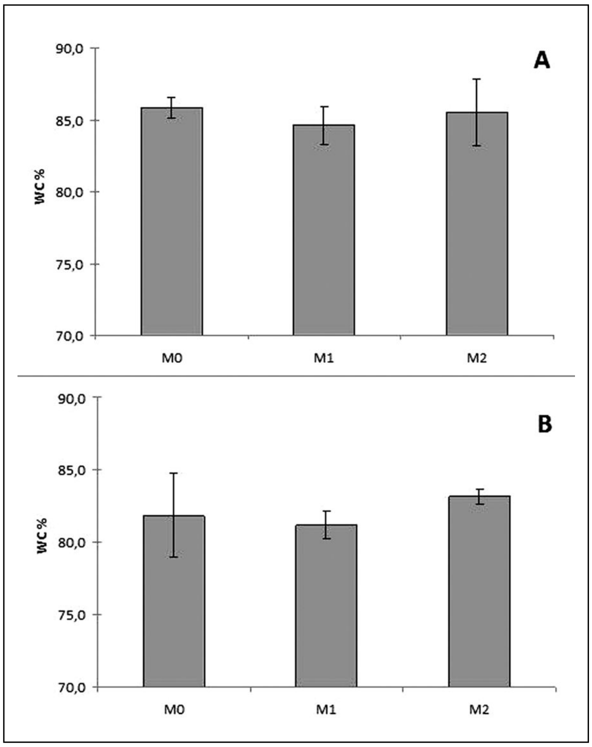

Fig. 8 - Leaf water content (WC) of maize plants treated with different microbial inocula (means±SD, *n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. A: water deficiency phase (14 days after irrigation); B: recovery phase (1 day after irrigation).
Fig. 8 - Contenuto idrico delle foglie (WC) delle piante di mais trattate con diversi inoculi microbici (media±DS, n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. A: fase di carenza idrica (14 giorni dopo l'irrigazione); B: fase di recupero (1 giorno dopo l'irrigazione).

The different composition of the microbial populations in the rhizospheric soil, that resulted in plants treated with both consortia with respect to uninoculated plants (Tab. 2), can be at least partially ascribed to the presence in the inocula of aerobic bacteria only. The behaviour of anaerobic culturable bacteria populations was different in comparison to aerobic species: the plants inoculated with the commercial microbial consortium resulted to have a smaller population of anaerobic bacteria in comparison to not inoculated plants (Tab. 2). Several different reasons linked with the modification of rhizosphere conditions from both the plant and the environment could be hypothesized for this result. An increased respiration rate of roots with consequent reduction of oxygen availability would be possible considering the higher growth of not inoculated roots (Fig. 7). This is confirmed by several reports which are showing that oxygen is very actively consumed in the rhizosphere due to root respiration and the reducing power is a long observed property of plant roots (Uren, 2007). Also the significant difference in the rhizospheric population of fungi and yeasts between inoculated and control plants (Tab. 2) is an interesting trait of a more general modification of the rhizosphere microbial populations which could be driven by the plant (Hartmann *et al.* 2009). The matric potential of rhizosphere, which determines the distribution of water-filled pores and which consequently can alter the diffusion rates of gases to and from microbial populations, might regulate the activity of aerobic against anaerobic organisms (Young and Ritz, 2000).

As expected, the inoculation with AM fungi species resulted in an increase in the rate of root colonization, irrespective of the consortium utilized; indeed, AM fungi are generally not plant-specific (Read and Smith, 2008). However, the dynamic of colonization of the maize roots resulted in a higher rate for plants inoculated with the commercial inoculum in comparison to the one containing species selected from the Senegalese soil (Fig. 9). Such result could point out a certain degree of specificity, since the species of the M1 inoculum were selected from tomato rhizosphere in a sahelian environment, while the commercial inoculum contained species selected from plants and

	N	**P**	**K**	**Mg**	**Ca**
M0	2.926 ± 0.069	0.332 ± 0.080	2.304 ± 0.324	0.227 ± 0.026	0.399 ± 0.116
M1	3.235 ± 0.401	0.352± 0.069	2.233 ± 0.225	0.308 ± 0.060	0.672 ± 0.238
M2	3.337 ± 0.144	0.345 ± 0.075	2.189 ± 0.175	0.261 ± 0.064	0.481 ± 0.144

Tab. 1 - Mineral content of leaves of maize plants treated with different microbial inocula (% dry weight - means±SD, *n*=3). N: nitrogen, P: phosphorus, K: potassium, Mg: magnesium, Ca: calcium. M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium.
Tab. 1 - Contenuto in elementi minerali delle foglie delle piante di mais trattate con diversi inoculi microbici (% peso secco - media±DS, n=3). N: Azoto, P: Fosforo, K: potassio, Mg: magnesio, Ca: calcio. M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale.

	Aerobic Bacteria	Anaerobic Bacteria	Yeasts and Fungi
M0	1.31± 0.7 * 10^5	5.81± 0.4 * 10^5 a	4.31± 0.02 *10^4 a
M1	2.53± 0.1 * 10^5	5.74± 0.7 * 10^5 ab	1.10± 0.3 * 10^4 b
M2	3.39± 1.9 * 10^5	4.75± 0.08 * 10^5 b	2.26± 0.7 * 10^4 ab

Tab. 2 - Cultivable microbial populations of rhizospheric soil of maize plants treated with different microbial inocula (CFU · g^{-1} soil dry weight). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium. (Means±SD, *n*=4). Means with different letters are significantly different at P≤0.05 according to LSD test.

Tab. 2 - Popolazioni microbiche coltivabili del suolo rizosferico delle piante di mais trattate con diversi inoculi microbici (CFU • g^{-1} peso secco di suolo). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale. (Medie ± SD, n=4). Medie con lettere diverse sono significativamente differenti per P ≤ 0,05 secondo il test LSD.

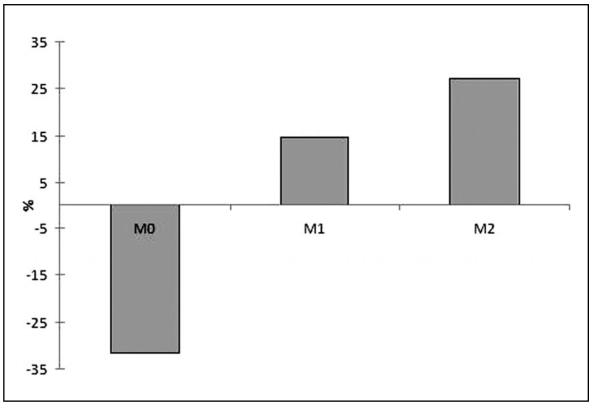

Fig. 9 - Roots AMF colonization of maize plants treated with different microbial inocula. Bars represent the percent variation between the rate of roots colonized after 100 and 40 days after sowing (*n*=3). M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium; M2: plants inoculated with commercial rhizospheric consortium.

Fig. 9 - Colonizzazione con funghi micorrizici delle radici delle piante di mais trattate con diversi inoculi microbici. Le barre rappresentano la variazione percentuale tra il tasso di radici colonizzate dopo 100 e 40 giorni dalla semina (n=3). M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità; M2: piante inoculate con consorzio rizosferico commerciale.

soil conditions more similar to those used and applied in the trial, thus confirming the outcomes from other studies suggesting the existence of some preferences between host plants and AM fungi (Helgason *et al.*, 2002; Stutz *et al.*, 2000).

Considering globally our results, the inoculated plants showed an increased association with both AMF and rhizosphere bacteria with respect to control plants; these results suggest that the selected consortia were effective in colonizing the rhizospheric soil, even if they were not composed of autochthonous species. This effect is sought with the application of rhizosphere technologies and has been shown to sustain both growth, yield and health of inoculated plants (Vessey, 2003; Raaijmakers *et al.*, 2009).

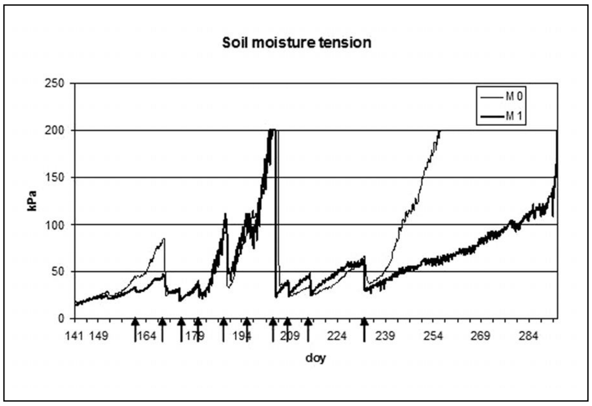

Fig. 10 - Dynamic of the soil moisture tension of microcosms during the experiment. Arrows indicate the irrigation events. M0: control plants (without microbial inoculum); M1: plants inoculated with drought stress-selected rhizospheric consortium.

Fig. 10 - Dinamica della tensione idrica del terreno dei microcosmi durante l'esperimento. Le frecce indicano i momenti in cui sono state effettuate le irrigazioni. M0: piante di controllo (senza inoculo microbico), M1: piante inoculate con consorzio rizosferico selezionato per lo stress da siccità .piante inoculate con consorzio rizosferico commerciale.

Inoculation with the microbial consortia modified consistently and deeply the gas exchange characteristics of the plants. Leaf stomatal conductance, transpiration rate and CO_2 uptake were all increased in inoculated plants in comparison to non inoculated (Fig. 2, 3, 4). These results are confirming several earlier studies, showing a significant impact of mycorrhizal fungi on the physiology of the plant not only at the root level (Augé, 2001). In our trial, the inoculated plants were better responding to the water deficiency stress not only during the occurrence of the stress, but also in the recovery phase. In the latter phase, the plants inoculated with the microorganisms selected from the arid environment were showing a significantly higher value of the measured leaf gas exchange parameters, which is indicating a faster recovery of the plants to normal physiological conditions and a better plant performance/survival in the conditions of water deficiency stress.

A higher g_s rate in M1 and M2 plants could derive from a more efficient extraction of soil moisture by mycorrhized root system in dry soil. The previous studies have suggested that extraradical hyphae (Allen, 1991) or increased root branching (Kothari *et al.*, 1990) may allow mycorrhizal roots to more fully explore a particular soil volume and increase the access to available water. Mycorrhizal symbiosis changed g_s of host plants, even when no changes in host plant size or phosphorous nutrition were recorded (Bethlenfalvay *et al.*, 1987; Augé *et al.*, 1992; Ruiz-Lozano *et al.*, 1995). Moreover, AMF can affect the point of stomatal closure during a soil drying period whether the host is efficiently avoiding or is highly tolerant to drought stress (Augé *et al.*, 1986 and 1992).

Considering gas exchange and growth data all together (g_s, E, A rate and root/shoot ratio), M1 and M2 plants under water deficiency conditions appeared less stressed than non inoculated plants. Such behavior or response has also been observed in mycorrhized soybean (Bethlenfalvay *et al.*, 1987), rose (Henderson and Davies, 1990), lettuce (Ruiz-Lozano and Azcòn, 1995) as well as in alfalfa (Sànchez-Diàz *et al.*, 1990) under water deficiency stress and it agrees with the idea that selected AM fungi may be even more important for plants under drought conditions (Sànchez-Dìaz *et al.*, 1990).

The use of different AMF species were shown to affect differently the maize plant water relations: inoculation with *G. intraradices* increased leaf gas exchange parameters (Subramanian *et al.*, 1995; 1997), while the use of other *Glomus* species did not (Osonubi 1994). These reports and our results are therefore confirming that AMF effects on stomatal conductance behaviour among different *Glomus* species can differ. Whether the differences are due to adaptation of strains to different environmental conditions has not been elucidated. However, our results suggest that such mechanism could be proposed. The effect of the other rhizosphere microorganisms (i.e. bacteria) on plant water relations is not fully understood. Several studies are reporting their influence on this physiological response of the plants (Alami *et al.*, 2000, Saleem *et al.*, 2007; Kang *et al.*, 2010; Yang *et al.*, 2009; Bardi and Malusa 2012). 1-aminocyclopropane-1-carboxylate (ACC) deaminase-producing PGPR promote the plant growth by reducing the high ethylene concentration inside the plant that is caused by abiotic stresses. PGPR can also indirectly improve the plant response to drought stress by promoting root development, increasing root surface area or increasing number of root tips, due to the production of phytohormones such as IAA (Yang *et al.*, 2009, Arzanesh *et al.*, 2011). Sandhya *et al.*, (2010) found that inoculation of maize seeds with *Pseudomonas* spp. under drought conditions improved plant biomass, relative water content, leaf water potential, roots adhering soil/root tissue ratio, soil aggregates stability, mean weight diameter and decreased leaf water loss; inoculation with PGPR lowered the activity of antioxidant enzymes, such as ascorbate peroxidase, catalase and glutathione peroxidase, indicating that inoculated plants were less stressed.

The bacterial strains selected and used in the M1 consortium were identified as *Bacillus subtilis* and *Halomonas* sp. *Bacillus subtilis* is well known as PGPR (Lugtenberg and Kamilova, 2009). A *Halomonas* sp. strain was recently isolated from *Salicornia brachiata* rhizosphere and characterized as PGPR; this strain possess a ACC deaminase gene, described for the first time in *Halomonas* sp., that not show homology with any *acdS* gene available in NCBI databases (Jha *et al.*, 2011). It could be suggested that the more positive effect of the M1 consortium in comparison to the commercial one could be ascribed to the presence in the former of bacteria specifically selected for their tolerance to osmotic stresses.

Even if no significant differences were observed in water use efficiency in our work, a different effectiveness of the two microbial consortia could be hypothesized considering the data of the other parameters. In fact, M2 plants during the recovery phase showed a decrease of stomatal conductance and transpiration rate, while leaf water content was slightly higher than in M0 and M1 plants. This could be due to a better ability of M2 plants in saving water content reducing the transpiration rate: the percentage of water content lost during recovery was lower (2.39%) than in M1 (3.45%) and M0 (3.97%) plants (Fig. 8). Water use efficiency has been reported to be lower when water availability is higher (Pou *et al.*, 2008). This could explain the decrease in water use efficiency of M2 plants during recovery (Fig. 5). On the contrary, M1 plants showed an increase of stomatal conductance and transpiration rate and a decrease of leaf water content under recovery, accompanied by a water use efficiency slightly higher than in M2 plants. Stomatal conductance can be higher in mycorrhizal than in non-mycorrhizal plants at similar low soil water potential (Auge *et al.*, 1987). A possible explanation for higher stomatal conductance in mycorrhized plants at low soil water tension could be due to a more efficient extraction of soil moisture by the mycorrhizal root system in dry soil or to the water uptake from the extraradical hyphae or increased root branching (Duan *et al.*, 1996; Kothari *et al.*, 1990) that may allow mycorrhizal roots to more fully explore a particular soil volume, and giving to the mycorrhizal root system more access to available water. We have not evaluated the changes in the root architecture after inoculation, and no differences in the root biomass were measured between the inoculated and untreated plants. However, we cannot dismiss the possibility that the bacteria strains of the two consortia, through the growth promotion effect on the roots, had modified the branching of the roots, which would also account for the higher conductance.

The different effects on stomatal conductance among different consortia could be explained from an ecological or a plant metabolic point of view. In fact, stomatal closure either when water is shortened (ecological reasons) or when plant metabolism decreases and a feedback signal of high CO_2 in the leaf drives stomatal closure. Upon water stress, sub-stomatal CO_2 concentration in inoculated plants was lower (one third) than in non inoculated controls (Fig. 6, A), showing that better hydraulic performances of inoculated roots ameliorated plant metabolism linked to water availability, and under these conditions no feedback signal is expected to close stomata. After irrigation, sub-stomatal CO_2 concentration did not change in M0 plants, whereas it was 2.5 times higher in M1 ones, reaching levels of M0 plants, and it was 5 times higher in M2 plants (Fig. 6, B). We hypothesize that in M2 plants a metabolic stomatal limitation occurred after irrigation, affecting negatively water use efficiency. M2 plants loosed water without gaining in carbon uptake, which accumulated and forced stomata to close.

The inoculation with both consortia resulted in a higher content of total nutrient elements in the plants even though the differences for the individual elements were not significant (Table 1). In dry soils, roots may lose their functions due to desiccation, thus reducing the uptake of nutrients. The uptake is also hampered by the reduced nutrient mobility. The presence of AM symbiosis, increasing the volume of soil explored and modifying the plant water relationships, is thus assuring a higher availability and uptake of mineral nutrients. In our experiment, as in others (Masia *et al.*, 1994; Duan *et al.*, 1996), the concentration of Ca in leaves did not change predictably in response to water stress in M0, M1 and M2 plants. However, it has been reported that AMF inoculation changed root and leaf concentrations of calcium (Augè *et al.*, 1992), suggesting its putative role in root-to-shoot communication of changes in soil water content. Inoculation of maize plants with *G. intraradices* showed an increase in N leaf content in drought stressed plants (Subramanian and Charest, 1998), but changes of the other elements had not always towards an increasing trend (Subramanian *et al.*, 1997). Even though in our experiment, the amount of N in the leaves was higher in inoculated plants, no effects were found on the chlorophyll content estimated on the basis of the chlorophyll index (Fig. 1). Leaf greenness quantified by the SPAD chlorophyll meter represents a well established relative measure of leaf chlorophyll content in maize (Dwyer *et al.*, 1991). Therefore, it appears that the functioning of the photosynthetic apparatus was not negatively affected by the water stress experienced by the plants.

Soil moisture was significantly conditioned by M1 inoculum; in fact, moisture tension rose very slowly after the last irrigation in M1 inoculated mesocosms in comparison to M0 mesocosms (Fig. 10). This can be explained by an improvement in soil structure, through deposition of organic compounds such as glycoproteins or exopolysaccharides due to the microbial activity, with the increase of water retention as a consequence.

The overall effect of the inoculation with the beneficial microrganisms on plant biomass was positive, inducing an increased production of biomass, but not statistically significant (Fig. 3). However, it must be recognized that plants inoculated with the consortium of microorganisms selected from the arid soil produced on average higher biomass by 30% than the control and 10% higher than the plants inoculated with the commercial consortium. The latter showed also an average 20% increase in total biomass production in comparison to non inoculated plants. Celebi et al (2010) evaluated the effect of the addition of AMF to soil under different irrigation regimes on the silage maize yield. A positive effect of AMF on growth and yield was observed in all the irrigation regimes analyzed, but in particular when the lowest level of water was provided (soil moisture kept at 4%). Our work was carried out at mesocosm level and water was provided to plants every two weeks. The strongly positive effect observed on the physiology of the inoculated plants, in particular when the specifically selected consortium M1 was applied, makes it possible to foresee a probably positive effect also on the biomass yield under field conditions and limited water availability. This hypothesis is currently tested on a field trial carried out with the same maize hybrid and the same drought-selected rhizospheric consortium used in this work. Indeed, even though an increasing plant traits of experimental evidence is pointing out that the appropriate management of microbial activities allows to enhance plant growth and health (Jeffries *et al.*, 2003; Vessey 2003). It is still difficult to predict the outcome of interactions between plants and beneficial soil microorganisms under field conditions since rhizosphere ecology, plant physiology as well as technological issues related to microbial delivery are all affecting the efficacy of the inoculation (Malusà *et al.*, 2012; Lucy *et al.*, 2004).

In conclusion, the use of rhizospheric microorganisms consortia specifically selected to adapt to arid environments and to improve drought stress tolerance of cultivated plants appears to be a potentially powerful and cheap tool to improve adaptation to climate change in agriculture, to obtain good crop yields under conditions of low water availability. Such approach could achieve food self-sufficiency of water-deficient underdeveloped countries and reduce the environmental impact of agricultural productions in high-water consuming developed countries.

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Extreme rainfall in the Lombardy region

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Abstract: *The climatology of extreme precipitation in Lombardy has been based on 1951 – 2005 daily data as reference period on the base of a network of 30 rain gauges characterized by data availability higher than 95% of total data. Extreme events have been detected dividing the daily rainfall into pre-defined classes (<20 mm "Weak", 20÷50 mm "Moderate", 50÷100 mm "Heavy", and >100 mm "Extreme") and calculating, for the whole dataset, the mean contribution of each class to yearly totals (in terms of percent), defining the relevance of each class to the overall phenomena. In general the analysis shows that Lombardy is physiologically characterized by high precipitation events due to the co-operaton of causal factors like the closeness of a remarkable source of water vapour (the Mediterranean sea), the peculiar shape of the relief (Alps and Apennines) and frequency and persistence of suitable circulation patterns at macro and mesoscale. A trend test performed on the whole territory showed the absence of significative trends in the frequency of extreme events.*
Keywords: *precipitation, extreme rainfall events, climatology.*

Riassunto: *La climatologia delle precipitazioni estreme in Lombardia si è basata su dati giornalieri di una rete di 30 stazioni pluviometriche caratterizzati da una disponibilità dei dati superiore al 95% nel periodo di riferimento 1951 - 2005. Gli eventi estremi sono stati analizzati dividendo gli eventi precipitativi giornalieri in classi predefinite (<20 mm "debole", 20 ÷ 50 mm "moderato", 50 ÷ 100 mm "intenso", e > 100 mm "Estremo") e poi calcolando, per l'intero set di dati, il contributo medio di ogni classe ai totali annui (in termini di percentuale), definendo la rilevanza di ciascuna classe sul totale annuo. In generale, l'analisi mostra che la Lombardia è fisiologicamente caratterizzata da forti precipitazioni a causa dell'azione congiunta di fattori causali, come la vicinanza di una notevole fonte di vapore acqueo (il Mediterraneo), la particolare forma del rilievo (Alpi e Appennini) e la frequenza e la persistenza di tipi circolatori a macro e mesoscala. L'analisi di trend, eseguita sull'intero territorio, non ha evidenziato trend significativi nella frequenza degli eventi estremi.*
Parole chiave: *precipitazioni, eventi precipitativi estremi, climatologia.*

INTRODUCTION

Lombardy, located in the North of Italy (river Po basin) and landlocked between the Alps in the North and the Northern Apennines in the South, shows a transitional climate, between Oceanic (Koeppen Cfb) and Mediterranean (Koeppen Csa) (Koeppen and Geiger, 1936; Pinna, 1972). More specifically, Mediterranean effects prevail in the Southern Apennine belt (as confirmed by the relevance of summer drought), continental effects prevail in the Po plain (as attested by cold winters and hot-humid summers) and oceanic effects prevail the pre-alpine belt (as proved by mild temperatures with abundant precipitation along the whole year). Moreover the area of the Alpine massif is characterized by a typical mountain climate - Koeppen H (Barry, 1992).

In this peculiar context, rainfall changes with altitude and orientation of the mountain ranges and reaches maximum values on Pre-Alps, where annual precipitation locally exceeds 2000 mm. These phenomena are the result of the interaction of synoptic and mesoscale circulation patterns with the relief that gives rise to some peculiar dynamical effects (like foehn-stau, air-mass convergence in valley systems and seeder–feeder orographic enhancement of convection) widely discussed in the scientific literature (Andersson , 1981; Barry, 1992) and deeply studied in two specific international research projects ALPEX (WMO, 1986) and Mesoscale Alpine Programme (Binder and Schar, 1996).

Going downward to the lowland areas, rainfall gradually decreases, reaching about 800 mm per year, with an Eastern minimum (Apennines foothills) and a Western maximum (Mantova province), along the Po river, where rain reaches about 650 – 750 mm per year. It might be interesting to say that similar values are reached in the center of the Alpine massif (Eastern part of the Sondrio province) where the protection of high reliefs give rise to a peculiar rainshadow effect (Endo-alpine effect).

With regard to the rainfall regime, the precipitation peak is reached during summer in the Alps while in the Pre-Alps, in the Po plain and in the Apennines the maximum is reached in Autumn and Spring (absolute and secondary maximum respectively). The Winter season is the poorest of precipitation North of the Po river, while South

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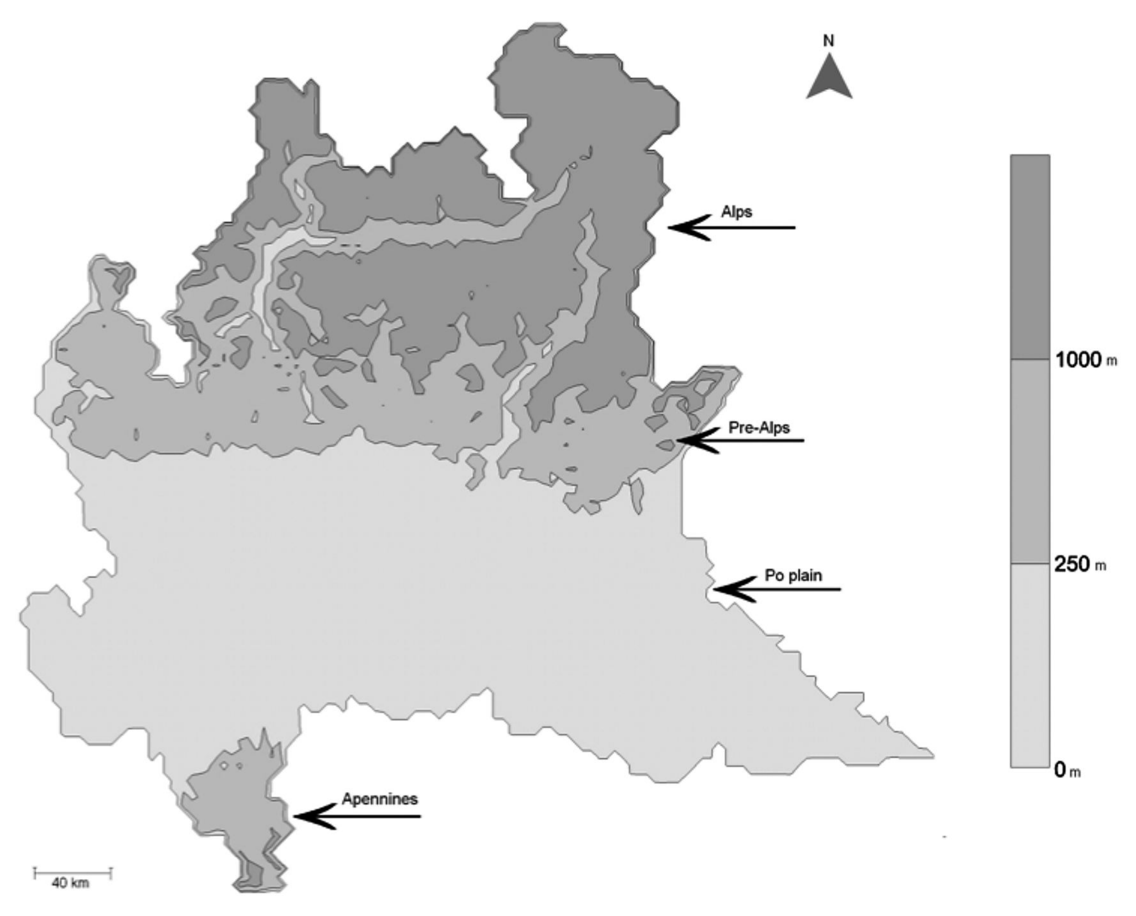

Fig. 1 - The four main mesoclimatic areas of Lombardy (Alps, Pre-Alps, Po plain, Apennines) and the three altitudinal limits are highlighted.
Fig. 1 - Vengono evidenziate le quattro principali aree mesoclimatiche della Lombardia (Alpi, Pre-Alpi, Pianura Padana, Appenini) e le tre fascie altimetriche.

of the Po river the absolute minimum is in summer.

Significant levels of precipitation are obtained only when the proper clouds, result of condensation processes on effective condensation nuclei, are present.

A cloud thickness of some thousands of meters and a sufficiently low cloud base are the two premises to trigger and sustain the microphysical mechanisms favorable to the growth of ice crystals or water droplets big enough to reach the soil and therefore induce significant precipitation. Conditions for clouds development are (I) the presence of an atmospheric dynamic environment favorable to air lift and (II) an air mass rich in water vapor rising from the boundary layer up to the free atmosphere. Both these factors are effective in Lombardy due to the steep orography, the closeness to the Mediterranean sea (a very effective source of water vapour, mainly coming from the Adriatic and Tyrrhenian Sea) and circulation patterns favorable to air lift. The main circulation patterns favorable to precipitation are:

– North Atlantic upper troughs that reach the area from West embedded in the westerly flow. When such troughs approach the Po valley from West a typical wind regime with an av erage direction East-West is triggered, sometimes associated with a low level jet (Buzzi *et al.*, 1998). The true direction of this flow determines the position of the horographic (staü effect) precipitation maxima that for instance could drop on Apennines or Prealps.

– Mediterranean cutoff lows generated by the interaction between troughs and mountains. The most influencing are Genoa Lows resulting from the interaction of the Atlantic flow with Alps and Apennines. Less frequent but sometimes effective are other Mediterranean lows like West African Lows or Tyrrhenian ones.

The theory behind air uplift is based on the dualism between troposphere and air parcels / layers (Mc Intosh & Thom, 1972). In the presence of an absolutely unstable thermal profile, the ascent of a generic air parcel occurs spontaneously by convection. In case of a potentially instable profile, the ascent usually occurs when the surface layer is bodily lifted up high enough to trigger condensation that provides the energy necessary for further development. The energy for the ascent of the surface layer comes from thermally forced local phenomena such as the land-sea breezes or upslope flows in mountain valley breezes. At larger scale, air uprising phenomena takes place as frontal or orographic air lift or dynamical convergence effects typical of some mesoscale phenomena (van Delden, 2001). Typically the ascent leads to cumuliform clouds if quick but local or leads to stratiform clouds if slow but widespread.

In the abovementioned aerological perspective it is important to consider the features (temperature, humidity, lapse rate and so on) of different air masses that comes from their long persistence on a given source region with peculiar characteristics. Therefore the air mass that dominates the low troposphere in the Po basin is very stable (Fea, 1988), which allows a strong storage of humidity that represents the main energy supply for thunderstorms which are triggered by outbreaks of Atlantic polar maritime air in the middle troposphere (in the Po basin, the thunderstorm season usually extends from the end of March to mid November).

Heavy precipitation events (HPEs) (whith Daily precipitation that exceeds 50 mm (Alpert et al, 2002) play an important role in Lombardy region and their periodic recurrence is an important element of climatic risk of landslides and floods. In Lombardy, during the most recent period phenomena of this kind affected the Apennine

Fig. 2 - The provinces of Lombardy and the Po river.
Fig. 2 - Le province lombarde e il fiume Po.

sector (October 1983), the Alpine area (July 1987), Western Lombardy (November 1994); Western Prealps (September 1995) and Central-Eastern Prealps (November 1996). Moreover an historical overview shows a recurrence of extreme erosive events in the Po basin along centuries, as stated for the period 1775-2003 by the analysis of the erosional process carried out by Diodato and Mariani (2007) and based on Milano Brera rainfall data.

So HPEs can be studied by the point of view of (I) causal factors (determinants at different scales approached by means of methods of atmospheric physics) (Giacobello and Todisco, 1979; Hoinka *et al.,* 2006), (II) phenomenological features (quantity, intensity and spatial distribution of phenomena approached with the methods of the rainfall meteorology and climatology) (Cantù, 1977) and (III) effects on surfaces (soil erosion, river floods and flash floods analyzed with tools of hydrology, geomorphology, geology and soil science) (Diodato and Mariani, 2007). The current analysis of HPEs will be limited to the first two approaches.

In this general context and focusing on the management purposes in agriculture and other socio-economic sectors it is important to analyze extreme precipitation events and related temporal trends. By this point of view, it can be observed that a widely adopted cliché after flooding events is that climate change is the responsible of the increase of extreme events. This statement was almost partially advocated by Alpert *et al.,* (2002) who spoke of "paroxystic increase of extreme events" in the Mediterranean and more specifically for Italy.

Nevertheless there is an increasing number of studies that dismiss the idea of "paroxystic increase" and, for instance:

– Polemio and Petrucci (2012) for the period 1880-2007 in Calabria, evidenced a decreasing in precipitation days, almost constant intensity, slight decrease in floods since the '70s.

– Polemio and Lonigro (2011), stated a decreasing trend in rainfall quantity and intensity associated to an increase of precipitation days for Puglia region on the period 1918-2006.

– Brunetti *et al.,* (2011), stated a decrease of high intensity precipitation, especially during winter, for Calabria region (1923-2006).

– Pinna (2012), focusing on the daily time series of abundant rainfalls (above 50 mm^{-1}) of Pisa and working on the subperiods 1922-1950, 1951-1980 and 1981-2010, stated only a slight average increase in the frequency of such events and in their contribution to total rainfall.

On the other hand:

– Brunetti *et al.*, (2004) claimed that the Italian climate is becoming warmer and drier due to a decrease of precipitation driven by the reduction in the number of wet days, as precipitation intensity displays a positive trend. Moreover, the authors claimed a tendency both toward an increase of heavy precipitation events and long dry spells.

– for Piemonte region, Bassi *et al.*, (2011), analyzing the time series of precipitation with length of 1, 3, 6, 12 e 24 hours gauged for the period 1930-2004 highlighted an increase of intensity in the most recent period (1994-2004) that counteracts the negative trend observed in the previous periods and is attributed by the authors to the changes occurred in the pluviometric network (from the traditional network of the Servizio Idrografico to the electronic network of ARPA Piemonte).

In the light of this, our paper aims to:

– analyse the trend of daily precipitation for four main classes of intensity and for the whole territory of Lombardy and three selected subareas of plain, hills/low mountain and middle/high mountain

– Estimate the consequences for land use and land management.

DATA AND METHODS

A climatology of extreme precipitation in Lombardy can be established on the base of pluviometric stations networks with a suitable spatial and temporal detail. In this context an important source of data is represented by the dataset gathered by the RICLIC Warm project collecting data from 107 stations belonging to the networks of Servizio Idrografico, Arpa Lombardia and Servizio Meteorologico dell'Aeronautica. These data were quality checked on the base of a subjective comparison with neighbors data. Values judged wrong were deleted.

The final dataset adopted for this work is composed of 30 stations (Tab. 1; Fig. 3) and was obtained excluding stations with a data availability below the 95% of data over the 1951 – 2005 reference period. This choice was made in order to avoid the adoption of procedures of reconstruction that, applied to rainfall, give relevant errors especially for extreme events due to their high spatial variability (Kim and Pachepsky, 2011). Mountain stations of the final dataset belongs only to Prealps and Alps because the Apennines stations did not meet the stated pre-requisites of quality. Another possible drawback of these series is given by the changes occurred in the rain gauge network (from the traditional network of the Servizio Idrografico to the electronic network of

Altitudinal class				**Gauss-Boaga system**	
	Station	**acronym**	**height**	**Longitude**	**Latitude**
Po plain (below 250 m asl)	Mantova	MANT	19	1638883	5001225
	Cremona	CREM	45	1580165	4998361
	Verona	VERO	50	1632043	5029186
	Codogno	CODO	58	1555232	5001799
	Lodi	LODI	80	1539410	5018346
	Ghedi	GHED	102	1603652	5031416
	Brescia	BRES	120	1595219	5043017
	Milano Brera	MIBR	147	1514932	5034831
Hills and low mountains (between 250 and 1000 m asl)	S. Francesco a Mese	MESE	281	1529178	5128111
	Sondrio	SOND	298	1565986	5113017
	Chiavenna	CHIV	333	1530635	5129294
	San Pellegrino Terme	SPEL	355	1551994	5075843
	Gandino	GAND	570	1570466	5073524
	Edolo	EDOL	655	1603498	5113791
	Garzeno	GARZ	670	1519269	5109025
	Vedeseta	VEDS	817	1541894	5082077
	Valbondione	VALB	890	1577924	5098464
	Valle Ratti	VRAT	915	1537134	5115993
	Le Prese	LEPR	950	1604312	5133867
	Lanzada	LANZ	983	1567276	5124399
Middle and high mountains (above 1000 m asl)	Case Pizzini (Armisa)	CASE	1060	1576924	5108107
	Vedello Centrale	VEDE	1060	1569661	5106714
	Bormio	BORM	1225	1605148	5146909
	Scais	SCAI	1500	1571278	5104281
	S. Caterina Valfurva	SCAT	1740	1615176	5140906
	Sardegnana	SARD	1750	1562145	5096309
	Lago Venina	VENI	1800	1566994	5104245
	Piano delle Casere	PCAS	1832	1561820	5094157
	Stuetta	STUE	1850	1526860	5146170
	Cancano	CANC	1930	1601307	5152415

Tab. 1 - List of stations adopted, subdivided into altitudinal classes.
Tab. 1 - Elenco delle stazioni utilizzate per questo lavoro suddivise in tre classi altitudinali.

the ARPA meteorological service), during eighties and nineties.

Furthermore, in order to highlight the temporal behavior of HPEs on secular periods the time series of Milano Brera (1858-2003) was analyzed. This series belongs to one of the oldest observatories of the World, founded in 1763. Furthermore Milano is located in the central part of the plain, almost equidistant between Apennines and Alps and its precipitation regime shows the co-existence of the Oceanic signal and the Mediterranean one. These elements justify the belief that this very long time series can contain some information about the behavior of extreme events processes of the whole Lombardy area (Diodato and Mariani, 2007). A possible drawback of the Brera series is given by the inhomogeneities due for example to changes in instruments (technology, exposure) and methods of observation. An analysis of this problem has been carried out by Clistovsky *et al.*, (1999).

In order to focus on HPE, for each of the 30 studied stations, rainfall daily events were divided into four classes with the same approach adopted by Alpert *et al.*, (2002): Weak (RR<20 mm), Moderate (20 mm< RR < 50 mm), Heavy (50 mm< RR < 100 mm) and Extreme (RR > 100 mm). Afterwards, the contributions of the four classes to the yearly total rainfall (expressed as percentage) were obtained in order to define the impact of each class on the overall phenomena.

Climate change can be considered as a significant change in the statistical properties of the climate system when considered over periods of decades or longer (Pielke and Waage, 1987; Arguez and Vose, 2011; WMO, 1986). Among statistical properties, particularly important for practical purposes are the indexes of central tendency (e.g.: arithmetic mean, median) and dispersion (e.g.: standard deviation, interquantile range).

The trend analysis of rainfall classes contribution to total yearly precipitations was performed with the Mann – Kendall test and the Sen's slope (Salmi *et al.*, 2002) in order to determine the trend and the slope magnitude. The Z test is useful to analyse the significance of trend . Sen's test is an estimator of trend magnitude. The Mann-Kendall test is based on the statistic S and it is dependent on the distribution type.

The importance of HPE was considered not only weighting the quantity (sum of precipitations belonging to a specific class) but also in terms of numbers of days with precipitation for each precipitation class.

RESULTS AND DISCUSSION

Tab. 2 shows the mean total number (and relative standard deviation) of daily events belonging to each precipitation class for the whole reference period (55 years) in each altitudinal class. Mountain stations have a higher probability of extreme events than Po plain stations. This shows the effectiveness of the enhancement on precipitation processes given by the Alps. In terms of risk for humans and goods it can be stated that the millennial work of extreme events shaped the alpine relief widening

Fig. 3 - Location of the 30 stations adopted for the analysis (for the meaning of acronyms see table 1).
Fig. 3 - Ubicazione delle 30 stazioni adottate per l'analisi (per il significato delle sigle vedi tabella 1).

Altitudinal class	Num stations	<20mm		20÷50 mm		50÷100 mm		>100 mm	
		Mean	***St.dev***	***Mean***	***St.dev***	***Mean***	***St.dev***	***Mean***	***St.dev***
<250	8	3806.3	553.6	476.6	69.3	50.4	7.3	1.8	0.3
250-1000	12	4209.8	918.5	750.4	163.7	162.3	35.4	12.5	2.7
>1000	10	4968.0	903.3	697.9	126.9	158.5	28.8	15.1	2.7

Tab. 2 - Number of precipitation days belonging to different classes for the reference period 1951 – 2005 (Avg is for the average number of total daily events for station in the reference period).
Tab. 2 - Numero di giorni con precipitazione appartenenti a classi diverse per il periodo di riferimento 1951-2005 (Mean rappresenta il numero medio del totale di eventi quotidiani per la stazione nel periodo di riferimento).

Time series	Time series	Test Z Signific.	Sen's slope estimate Q
Po plain (below 250 m asl)	<20 mm	-2.13*	-0.130
	20÷50 mm	0.77	0.041
	50÷100 mm	1.96*	0.082
	>100 mm	(++)	
Hills andlow mountains (between 250 and 1000 m asl)	<20 mm	-0.3	0.011
	20÷50 mm	-2.67**	-0.086
	50÷100 mm	1.54	0.061
	>100 mm	1.9	0.029
Middle and high mountains (above 1000 m asl)	<20 mm	-0.38	-0.020
	20÷50 mm	-0.58	-0.018
	50÷100 mm	-0.38	-0.016
	>100 mm	0.48	0.008
All stations	<20 mm	-0.84	-0.043
	20÷50 mm	-0.78	-0.023
	50÷100 mm	1.07	0.036
	>100 mm	1.51	0.015

Significance level: * is for > 90%; ** is for > 95%; *** is for > 99%
(++) The number of the events (only 14 on the period 1951-2005) is insufficient for the statistical analysis.

Tab. 3 - Trend analysis of the contribution of the four precipitation classes to total yearly precipitation. Data are referred to three altitudinal classes and the whole Region.
Tab. 3 - Analisi di trend del contributo delle 4 classi precipitative sul totale pluviometrico annuo. I dati si riferiscono alle tre zone altimetriche ed alla Regione nel suo complesso.

the valleys and removing a relevant part of the erodible debris. Nevertheless the relevant presence of extreme events is also today an important factor of climatic risk for alpine territories and must be taken into account by population and authorities that rules these areas in order to plan significant changes in land use.

Tab. 3 shows the result of the trend analysis carried out on precipitation belonging to the four reference classes for the whole territory and for the three reference subareas (plain, hills and low mountains and middle/high mountains.

The trend test for the whole territory, which is the most robust due to the high number of selected stations (30) and daily events, show the absence of trends. The same is true for the 10 stations of the middle and high mountains (above 1000 m asl). Conversely stations of the Po plain show a significant increase of "heavy" daily events (P>90%) and a significant decrease (P>90%) of "weak" daily events while stations of hills and low mountains show only a significant decrease (P>95%) for "moderate" daily events.

In order to interpret these data it is important to say that the robustness of the results obtained for higher intensities is negatively affected by the low number of events. This is particularly true for the "Extreme" class for the plain area, where the statistical analysis was unreliable due to the low number of events (only 0.3 events per year). Moreover, the number of events for this class is low also for low mountain and high mountain areas (2.7 events per year in both cases). On one hand this induces a cautious view on such results and on the other hand suggests the use of the results referred to the whole area in order to obtain a more robust interpretation of the trend of strong precipitation.

It is also intriguing to observe that the analysis for the four classes of events for the long time series of Milano Brera shows the total lack of significant trends in the four considered classes (Tab. 4) which

Time series	Test Z Signific.	Sen's slope estimate Q
<20 mm	0.33	0.006
20÷50 mm	-0.51	-0.008
50÷100 mm	0.38	0.003
>100 mm	1.29	0.001

Significance level: * is for > 90%; ** is for > 95%; *** is for >99%

Tab. 4 - Trend analysis of the contribution of the four precipitation classes to total yearly precipitation for Milano-Brera (1858-2003).

Tab. 4 - Analisi di trend del contributo delle 4 classi precipitative sul totale pluviometrico annuo, effettuate sulla stazione pluviometrica di Milano-Brera (1858-2003).

in our opinion give relevant information for the management of the strongly anthropised province of Milan.

CONCLUSION

This work, carried out on Lombardy area with the same methodology adopted by Alpert *et al.*, (2002), dismiss the hypothesis of the "paroxystic increase of extreme events", highlighting a substantial stability for the whole regional territory. These results confirm the ones obtained in other Italian areas (Polemio and Lonigro, 2011; Brunetti *et al.*, 2011; Pinna, 2012; Bassi *et al.*, 2011).

The only significant trends affect the Po plain for daily events of 50-100 mm (significant increase with P>90%) and for daily events below 20 mm

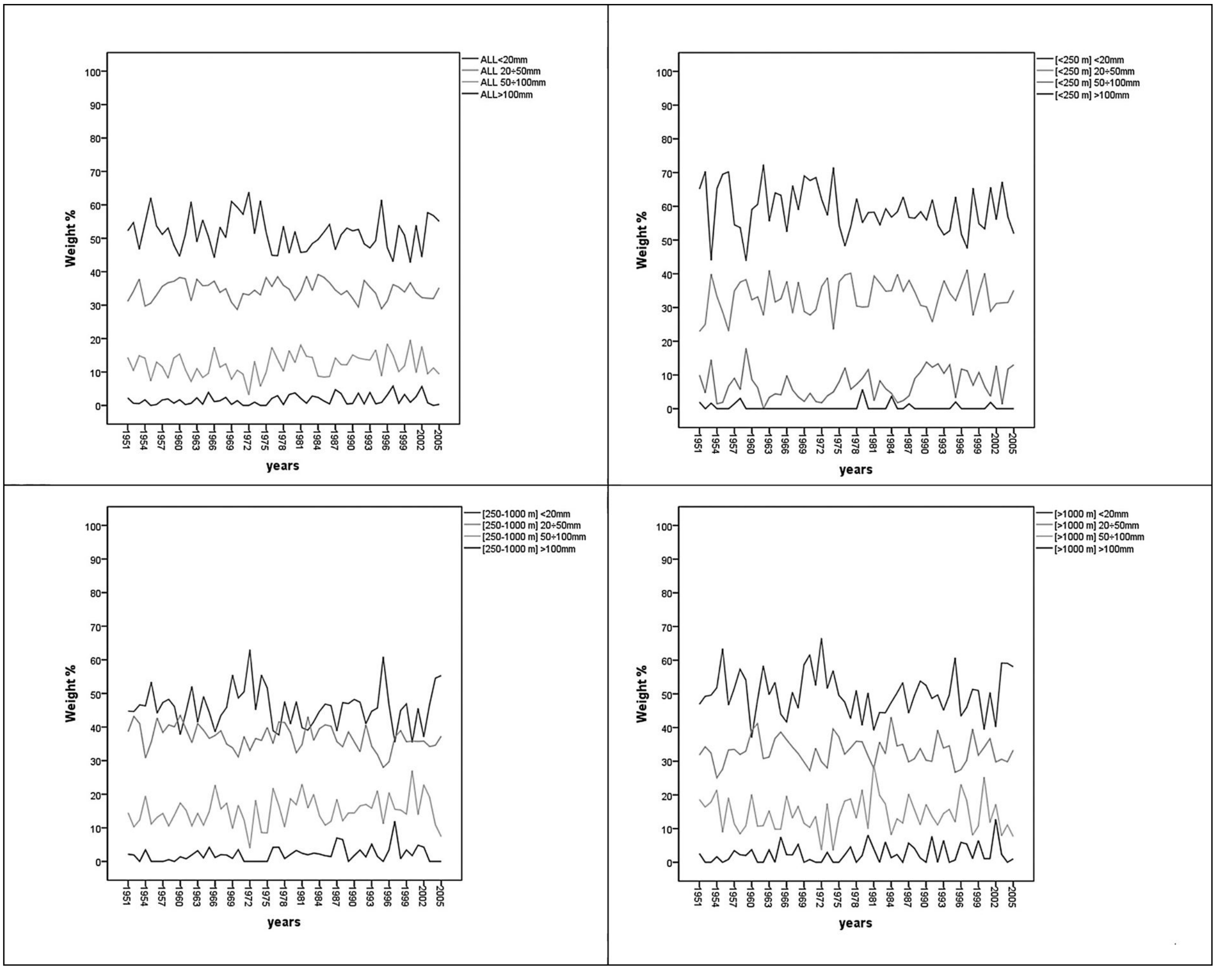

Fig. 4 - Behavior of mean contribution of the four precipitation classes to total yearly precipitation. The upper-left chart is referred to all the station, the upper-right chart to station located below 250 m asl. Lower-left and lower-right charts are referred to stations between 250 and 1000 m and above 1000 m asl respectively.

Fig. 4 - Andamento del contributo delle 4 classi precipitative sul totale pluviometrico annuo. L'andamento regionale complessivo è presentato nel grafico in alto a sinistra, mentre l'andamento delle stazioni posizionate al di sotto dei 250 m s.l.m. è presentato in quello in alto a destra. I grafici in basso a sinistra ed a destra presentano rispettivamente l'andamento delle stazioni comprese fra i 250 ed i 1000 m s.l.m. e quelle oltre i 1000 m s.l.m.

(significant decrease with P>90%) and hills / low mountain for daily events of 20 – 50 mm (significant decrease with P>95%). Moreover results referred to classes above 100 mm can be analyzed with caution due to the low number of events analyzed. by this point the more conservative analysis referred to the whole territory can be considered more realistic.

These views should lead to a more objective evaluation of extreme rainfalls based on reliable observational networks, systematic quality control and analysis undertaken for the whole country and homogeneous subareas.

Our analysis shows that Lombardy is physiologically characterized by strong precipitation events due to the co-operation of causal factors like the closeness of a remarkable source of water vapour (the Mediterranean sea), the recurrent arrival of peculiar air masses, the strength and the peculiar shape of the relief (Alps and Apennines) and frequency and persistence of suitable circulation patterns at macro and mesoscale. This awareness invite to suitable land management practices of territory. For example farmers should adopt field surface water control to avoid the excess of water caused by heavy precipitation exceeding the infiltration and drainage capacity of soils following the rules described in the Italian textbooks of agronomy (Mariani *et al.,* 2013).

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RICLIC - WARM PROJECT Regional Impact of CLImatic Change in Lombardy Water

Resources: Modelling and applications http://www.riclic.unimib.it/

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A Mediterranean conifer under vegetation shift: seasonal changes of photochemical activity in *Cupressus sempervirens* (L.) and evidence of correlation with temperature models

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Abstract: *The Italian cypress (Cupressus sempervirens L.) is widespread in the entire Mediterranean region and reaches in the Province of Trento high latitude (46° 21' North) and elevation (about 985 m a.s.l.). The human range expansion of this cypress has taken place at the Northern margin of the range in Italy in recent decades, driven by ornamental planting in spite of climatic constraints imposed by low temperature. The aim of the present work was to investigate the effect of low temperature in cypress in a nursery of the Autonomous Province of Trento (North-Eastern Italy). Over a period of two and half years, maximum quantum use efficiency of photosystem II (F_V/F_M) was monitored in order to study the effects of air temperature on the photosynthetic efficiency of 99 different clones and to derive information about the potential distribution of cypress in this province. A significant positive correlation ($r = 0.64$, $p < 0.001$) was found between air temperature and F_V/F_M over the 29-month period and especially in spring and autumn. Minimum values of F_V/F_M were observed in winter. Moreover, in order to assess critical environmental factors for cypress, mean values of F_V/F_M were correlated with daily air temperature and temperature-based indices to identify the driving factors of the physiological changes along the annual cycle. The maximum value of correlation ($r = -0.85$, $p < 0.001$) was found with 10-day Cold Degree-Day Running Sums before sampling and with a threshold of 2.0 °C for daily minimum air temperature.*

Keywords: *chlorophyll fluorescence, PSII quantum use efficiency (F_V/F_M), low temperature, cold tolerance, Cupressaceae, conifer planting, conifer adaptation, climate change.*

Riassunto: *Il cipresso italiano (Cupressus sempervirens L.) è un specie ampiamente diffusa in tutta la regione mediterranea e raggiunge nella Provincia di Trento elevate latitudini (46° 21' Nord) e quote (circa 985 m s.l.m.). L'espansione di origine antropica per uso ornamentale di questa specie ha avuto luogo nei più recenti decenni, talvolta senza considerazione per le limitazioni naturali dovute alle basse temperature. Scopo del presente lavoro è stato quello di indagare l'effetto delle basse temperature sul cipresso allevato in un vivaio della Provincia Autonoma di Trento (Italia). La massima efficienza quantica del fotosistema II (F_V/F_M) del cipresso è stata monitorata per due anni e mezzo per studiare gli effetti della temperatura in 99 cloni diversi e per ricavare informazioni sulla distribuzione potenziale del cipresso in questa provincia. Si è trovata una correlazione significativamente positiva ($r = 0.64$, $p < 0.001$) tra la temperatura e l'efficienza fotosintetica sul periodo di 29 mesi e specialmente in primavera e in autunno. I valori minimi di F_V/F_M sono stati misurati in inverno. Inoltre, per identificare i fattori ambientali critici per il cipresso, i valori medi di F_V/F_M sono stati messi in correlazione con la temperatura media giornaliera e con indici termici per meglio identificare i fattori guida del cambiamento fisiologico nel ciclo annuale. Il massimo valore di correlazione ($r = -0.85$, $p < 0.001$) è stato trovato con una somma mobile di 10 giorni di gradi di freddo con soglia di 2 °C sulla temperatura minima.*

Parole chiave: *fluorescenza della clorofilla, efficienza quantica del fotosistema II (F_V/F_M), basse temperature, tolleranza al freddo, Cupressaceae, coltivazione conifere, adattamento delle conifere, cambiamento climatico.*

INTRODUCTION

Together with several meteorological forcing agents, such as radiation, water and nutrient availability, air temperature can play a relevant role in temperate climates as driving factor for the vegetative cycle of plants. It controls phenological development (Schwartz, 2003), particularly bud break and leaf senescence in deciduous species. It is also involved in hardening and de-hardening processes and regulates photosynthesis and evapotranspiration (Farquhar *et al.*, 2001). However, these processes are difficult to predict if, for the investigated species, the relationships between environmental factors and the rate at which photosynthetic capacity is impaired with the onset of winter and regained in the spring are unknown (Nippert *et al.*, 2004).

In evergreen species, air temperature acts on both chlorophyll concentration and efficiency (Ensminger *et*

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al., 2004; Montagnani *et al.*, 2004), controlling the capacity of photosynthetic systems and plant growth. In particular, the combination of low temperatures with high light intensity and low soil water availability is responsible for winter desiccation damages (Montagnani *et al.*, 2004). In this context the leaf winter-reddening, especially common in Cupressaceae, seems to be associated to a photoprotection effect (Hughes, 2011).

Again, low air temperature directly affects winter fluctuation patterns of tree stem diameter and indirectly the switch between dormancy and development in the following spring (Cocozza *et al.*, 2009; 2012).

Genotypic differences among evergreen species may allow cold-tolerant species to increase annual productivity as a result of longer photosynthetic seasons (Marshall *et al.*, 2001). Although several conifer species can maintain some photosynthetic capacity following exposure to low winter temperatures (Leverenz and Öquist, 1987; Weger *et al.*, 1993), other species undergo a distinct photochemical inactivation during the winter months (Westin *et al.*, 1995; Rose ad Haase, 2002). Cold-tolerant genotypes may be better adapted to photosynthesize when prolonged exposure to low temperatures is followed by warmer spells (Fracheboud *et al.*, 1999).

Rapid changes in abiotic factors occur over short distances, leading to major changes in the selection pressure acting on plant traits. One major concern in this context relates to the ability of long-lived species to cope with rapid climate change (Abrams, 2011). These changes, already observed also in the Southern Alps (Eccel, 2005, Di Piazza and Eccel, 2012), may have affected the fitness of tree species growing in presently marginal regions of their distribution area, such as Italian cypress (*Cupressus sempervirens* L.) in low-elevation Alpine areas. These environments provide an ideal experimental setting for investigating stress adaptation in cypress, as selection pressures along ecological gradients are increased by climate change (Pauli *et al.*, 2007).

Photosynthesis in cypress was found to be significantly inhibited by high irradiance over 1900 µmol m^{-2} s^{-1}(La Porta *et al.*, 2004), by aging (La Porta *et al.*, 2006), and by canker infection (Muthuchelian *et al.*, 2005a), even though molecular mechanisms are different in resistant and susceptible clones (Muthuchelian *et al.*, 2005b). Also low temperature can heavily affect photosynthesis in Italian cypress (La Porta *et al.*, 2005) as well as in other Cupressaceae (Weger *et al.*, 1993). Nevertheless, a change in climate, namely warming, would be expected to shift the distribution of cypress as plants expand in newly favorable areas and decline in increasingly hostile locations.

Italian cypress is a medium-sized evergreen tree that usually shows a narrow and conic crown and is widely cultivated in forest and as an ornamental plant. The native area of this species is the Eastern Mediterranean region (Turkey and some Greek islands) and Iran characterized by hot dry summer and mild winters, but along the centuries cypress was introduced in regions very far North from its native range. Nowadays it is distributed along all Mediterranean coasts, but also in other continents like California, Australia, New Zealand, and South Africa. The Pre-Alps, the Alpine foothills and valley bottoms of the Southern slope of the Alps are important areas of natural vegetation of cypress, where it has considerable potential for further spreading in marginal areas with suitable microclimate (Zocca *et al.*, 2008, Baldi *et al.*, 2012). In Trentino (Italian Alps), cypress was introduced long ago (Bagnoli *et al.*, 2009). The species is currently present within an altitudinal range from 66 m a.s.l. (Lake Garda) up to 985 m a.s.l. in the Fiemme Valley (Fig. 1). An inventory study performed in 2004-2005 recorded about 15.000 individuals higher than 4 m and larger than 5 cm diameter at breast height (DBH). They grow as single trees or in groups scattered along ca. 1900 sites (Zocca *et al.*, 2008), more than 90% at elevations ranging from 50 to 350 m a.s.l., with prevalent aspects S, SW and W (unpublished data). Growing as single trees, the cypress habitus itself might favor the higher aerodynamic exposure to air circulation and consequently trees experiences critically low temperatures in winter.

Recently, a multidisciplinary scientific research project (ECOCYPRE) was carried out to evaluate cold tolerance of cypress and to improve planting material by assessing clonal selections (Pedron *et al.*, 2009; Baldi *et al.*, 2011). Cold tolerance itself can indirectly protect the tolerant clones from cypress canker disease (*Seiridium cardinale*) because it can avoid or limit the entry of the fungal spores represented by small bark micro-wounds due to cold stress. In fact, these cold injuries are often hardly noticeable, but they are sufficient to weaken the plant and open the way for the canker agent (La Porta *et al.*, 2008) and numerous other parasitic diseases, which the trees could otherwise escape (Manion, 1991).

The exposure to multiple stress factors would increase the vulnerability of cypress.

By assessing the photosynthetic efficiency, the use of chlorophyll fluorescence has been tested for long term monitoring of cold tolerance of several conifer species, including Norway spruce (Lundmark *et al.*, 1988; Montagnani *et al.*, 2004), western red cedar (Weger *et al.*, 1993), lodgepole pine (Lundmark *et al.*, 1988), larch and dwarf mountain pine (Montagnani *et al.*, 2004), Scots pine (Lundmark *et al.*, 1988; Montagnani *et al.*, 2004; Repo *et al.*, 2006), and Phoenician juniper and

Aleppo pine (Martínez-Ferri *et al.*, 2004). The ratio of variable to maximal fluorescence F_V/F_M measures the proportion of open photosystem II centres and quantifies the efficiency of energy capture, generally termed the "maximum quantum yield of photosystem II" (Genty *et al.*, 1989). Leverenz and Öquist (1987) found a significant correlation (0.91) between quantum yield and F_V/F_M in Scots pine. Generally, quantum yield decreases with temperature in the proximity of 0 °C (Leverenz and Öquist, 1987), which indicates photoinhibition, a protective mechanism for dissipating excess energy.

In this frame, the aim of the present work was to investigate the effect of low air temperature on F_V/F_M, measured in a collection of 99 selected cypress clones. Genotypes able to tolerate cold temperatures show rapid photosynthetic recovery when temperatures rise in spring (Fracheboud *et al.*, 1999). Chlorophyll fluorescence was used to test suitable temperature-related parameters that respond conveniently to photochemical activity, and to screen the cypress population for frost tolerance. A spin-off of the investigation was the definition of a limiting thermal threshold for cypress distribution and the comparison with thermal thresholds in Trentino, where the species grows at the Northern edge of its range, due to occasionally unfavorable conditions in terms of recurrent low temperatures (Raddi and Panconesi, 1989; Zocca *et al.*, 2008). By this work, we want to assess the best combination of time-lapse, temperature threshold and temperature record for the photochemical response of Italian cypress to cold stress. We hypothesized that i) there are differences in F_V/F_M annual trends among clones and ii) F_V/F_M response to temperature is effective and clone specific.

MATERIALS AND METHODS

Research area and planting material

The trees were planted in an experimental plot located about 2 km North of Riva del Garda (Trentino, North-Eastern Italian Alps; 45° 53´55,56 Lat. N; 10° 50´13,80 Long E) at 105 m a.s.l. of elevation on a deep well-drained fertile mixed soil on a flat site in the bottom of the Sarca river Valley (Fig. 1). Plant material consisted in 99 clones propagated by graft on wild cypress rootstocks. After 3 years growing in pots, ca. 1 m plants were transferred from the nursery to the experimental plot. Ten ramets for each clone were planted in randomized blocks. Each of the blocks contained one ramet of the 99 different clones. The clones represented the result of a 30 years breeding programme for *Seiridium cardinale* resistance carried out at the Research Institute IPP-CNR (Firenze, Italy) and kindly provided for the experiment trial.

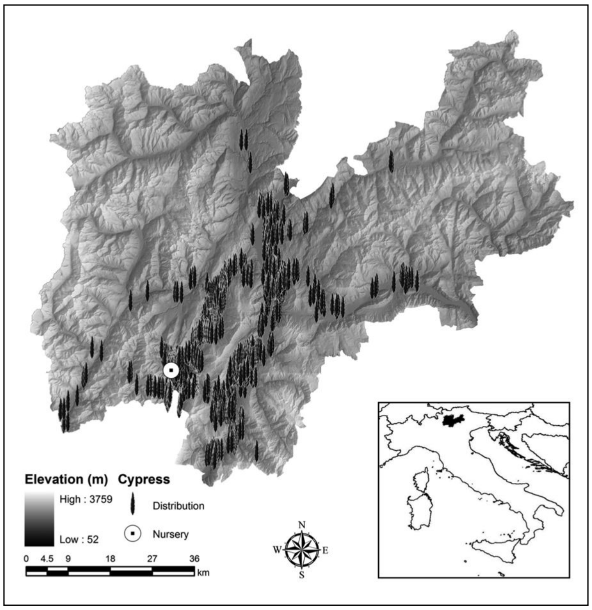

Fig. 1 - Distribution map of *Cupressus sempervirens* (L.) in Trentino (North-East Italy) and location of the nursery.
Fig. 1 - Mappa di distribuzione di Cupressus sempervirens *(L.) in Trentino e posizione del vivaio.*

Climatic and micrometeorological conditions

Main meteorological features for the site were derived by the Arco meteorological station located about 4 km away from the nursery, at 83 m a.s.l. The station, managed by the FEM CTT-SIG Unit, has been operating since 1983.

Lake Garda and the lower reach of the Sarca River have the lowest elevation among all the South-Central Alps (69 m a.s.l.). The presence of such a large water body in the outlet of the valley into the Po Plain is at the base of regional climatic peculiarities, mainly due to the presence of a regular breeze regime, known as "Ora del Garda". From a general point of view, the climate of the area can be classified as temperate, with no dry season ("Cfb" according to Köppen). In spite of its supposed sub-Mediterranean characters, the climate of Riva del Garda is classified as "humid", like all the low-elevation southern Alpine valleys. However, basing classification uniquely on temperatures and not on wind may be misleading in an area where the latter represents an outstanding climatic feature, and a more reliable framing of humidity/drought regimes should be shifted a little towards a drier class.

Temperature measurements close to the trees were carried out by two Tinytag Ultra data-loggers (Gemini Data Loggers Ltd., Chichester, UK), equipped with a shielded temperature probe. The loggers were placed at 150 cm height among the trees of the experimental plot and were programmed to record hourly temperatures. Data were averaged between the two loggers.

Parameter	**Expression**
Previous day minimum air temperature	$T_{min} = \text{Minimum}\ (T_{air})_{i-1}$
Previous day mean air temperature	$T_{mean} = \text{Average}\ (T_{air})_{i-1}$
Previous day maximum air temperature	$T_{max} = \text{Maximum}\ (T_{air})_{i-1}$
(3-5-7-10) -days running mean of T_{min}	$T_{min}\ RM_{\ n+1} = \sum_{1}^{i-n} T_{min} \quad n = 2, 4, 6, 9$
(3-5-7-10) -days running mean of T_{mean}	$T_{mean}\ RM_{\ n+1} = \sum_{1}^{i-n} T_{mean} \quad n = 2, 4, 6, 9$
(3-5-7-10) -days running mean of T_{max}	$T_{max}\ RM_{\ n+1} = \sum_{1}^{i-n} T_{max} \quad n = 2, 4, 6, 9$
(3-5-7-10-12-15) -days cold degree -day running sum of T_{min} lower than T_S (-5 to 25 °C)	$T_{min} CDDRS_{T_S} = \sum_{1}^{i+n} \min\ [T_S - T_{min}; 0] \quad n = 2, 4, 6, 9, 11, 14$
(3-5-7-10-12-15) -days cold degree -day running sum of T_{mean} lower than T_S (-5 to 25 °C)	$T_{mean} CDDRS_{T_S} = \sum_{1}^{i+n} \min\ [T_S - T_{mean}; 0] \quad n = 2, 4, 6, 9, 11, 14$
(3-5-7-10-12-15) -days cold degree-day running sum of T_{max} lower than T_S (-5 to 25 °C)	$T_{min} CDDRS_{T_S} = \sum_{1}^{i+n} \min\ [T_S - T_{max}; 0] \quad n = 2, 4, 6, 9, 11, 14$

Tab. 1 - List of the micrometeorological parameters and indices (running sums) used to correlate the quantum use efficiency data and to assess the critical temperatures for cypress physiology.
Tab. 1 - Lista dei parametri micrometeorologici e degli indici (medie mobili) da correlare con l'efficienza quantica e per valutare le temperature critiche per la fisiologia del cipresso.

Collection of shoots and needles

Sampling was performed monthly in the first period of the experiment (January 2004 – September 2005) and biweekly in the second one (October 2005 – May 2006). For each clone, an amount of 20-30 g of apical shoots was collected from the South-exposed part of the crown at 1.3 m from the ground. To avoid the negative effect of recurrent cuttings, different trees of the same clone were selected in different block each time. The sampled leaves were wrapped in aluminium foil, packed and sealed in a plastic envelope, in order to avoid dehydration. All the envelopes were placed into a portable cooling box and brought to the plant physiology laboratory. The measurements were performed within two hours after cutting. The shoots were dark adapted at 20 °C for at least 1 h before the fluorescence measurements.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured at room temperature using a modulation fluorometer (PAM-2000 Portable Chlorophyll Fluorometer Walz, Effeltrich, Germany). Fully developed shoots were fastened in the leaf clip, which maintains a fix angle and an adjustable distance from the leaf surface to the end of the optic fibre cable. This distance was kept constant at 5-7 mm during all measurements. The leaves were exposed to a 0.8 s saturated flash of approximately 6000 μmol m^{-2} s^{-1} to obtain the maximum fluorescence, F_M. The ratio between variable and maximum fluorescence, F_V/F_M, was calculated automatically according to measured minimum and maximum fluorescence yield, F_0 and F_M respectively. All measurements of F_0 were performed with the measuring beam set to a frequency of 600 Hz, whereas all measurements of F_M were performed with saturating flash automatically switching to 20 kHz. The F_V/F_M ratio $[F_V/F_M = (F_M – F_0)/ F_M]$ was used as a measure of the potential quantum yield. For each clone, chlorophyll fluorescence measurements were repeated on three shoots.

Environmental parameters

For the assessment of critical environmental parameters for cypress, the mean values of quantum use efficiency were correlated with the corresponding daily air temperature data and temperature-based indices in order to identify driving factors of physiological changes. Based on previous works (Bergh *et al.*, 1998; Montagnani *et al.*, 2004), for each sampling day (i) the values of many parameters for the day before (i-1) were retrieved or calculated from daily air minimum, maximum and average temperature (Tab. 1).

Running means were used to smooth out daily variability and to provide a clear trend line. Trials were done by varying length of the averaging period, 3 to 10 days before the sample collection, and using minimum, mean and maximum daily values of air temperature.

The number of days (n+1) of moving sum ranged from 3 to 15 while TS ranged between -5 and 25 °C. Cold Degree-Day Running Sums (CDDRS) were calculated based on Kira's coldness index (Kira, 1945; Krestov and Nakamura, 2007) using minimum, mean and maximum daily air temperature below a threshold temperature (TS).

Statistical analysis

Simple linear correlation analysis between air temperature-based indices (Table 1) and the mean values of F_V/F_M of all cypress clones was performed using the software Statistica 8.0 (StatSoft Inc., Tulsa OK, USA). Pearson correlation coefficients (r) and the significance level (p) were calculated for each micrometeorological parameter.

RESULTS

Climate and micrometeorology

The average annual air temperature of the nursery area over the last 25 years was 12.7 ± 0.1 °C, a rather high value, for a site within the edge of the alpine region, and is due to the low elevation of the station (70 m a.s.l.). The coldest month is January (3.0 ± 0.1 °C) while the warmest is July (22.7 ± 0.1 °C). The pluviometric regime, indeed, cannot be thought of as a "quasi-Mediterranean" one, since neither a true dry summer nor a rainy winter occurs. On the contrary, winter is the driest season (Fig. 2), while maxima of yearly rain supply are mostly recorded in autumn (principal maximum) and in spring (secondary maximum). The average yearly precipitation amount is about 830 mm.

The main micrometeorological parameters were similar during the two years of observation (Fig. 3). In 2004 and 2005 the mean value of air temperature was 13.1 °C and 12.6 °C, respectively, absolute minimum air temperature -5.4 °C and -8.9 °C, absolute maximum air temperature 36.5 °C and 33.2 °C. Yearly precipitation amount reached 980 and 845 mm, respectively.

Chlorophyll fluorescence

F_V/F_M showed a typical seasonal variation with a maximum in summer and minimum values in winter for all 99 clones (Fig. 4). The lowest mean values of F_V/F_M occurred in February 2005. Cluster analysis failed to recognize significant well-separated groups. Clone 1 and 31 showed the largest Euclidean distance (Fig. 4). On the contrary, interannual variability was high.

Correlations between environmental parameters and F_V/F_M values

Based on the air temperature data collected at the nursery, the daily values of the micrometeorological parameters and indices (Tab. 1) were calculated and

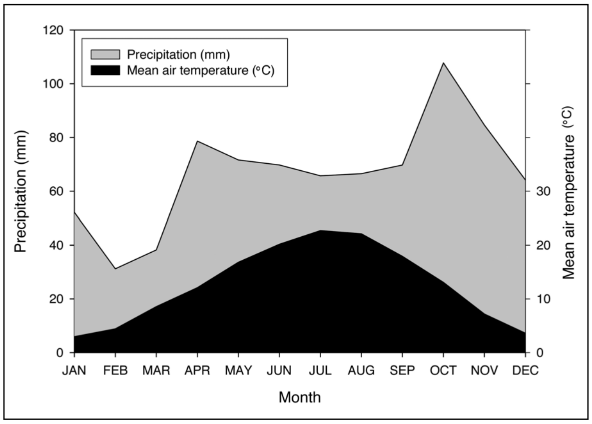

Fig. 2 - Bagnouls-Gaussen's diagram representing both mean monthly temperature and precipitation in conventional scales, where 1 °C corresponds to 2 mm of rainfall (data from the meteorological station of Arco; time series 1983-2008).

Fig. 2 - Diagramma di Bagnouls-Gaussen che rappresenta la temperatura media mensile e le precipitazioni in scala convenzionale, dove 1 °C corrisponde a 2 mm di precipitazione (dati della stazione meteorologica di Arco; periodo 1983-2008).

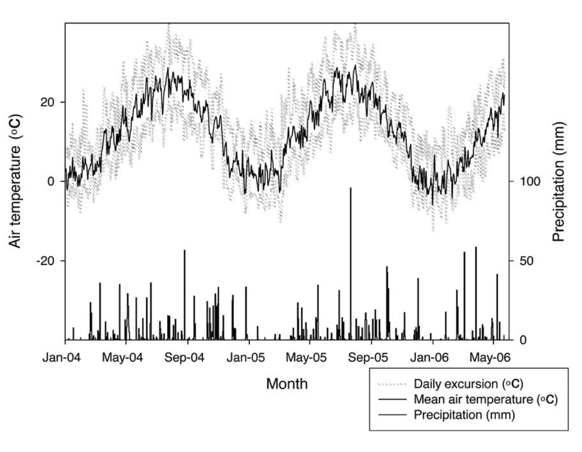

Fig. 3 - Two-and-a-half-year trend of daily mean air temperature, thermal range and *precipitation at the Arco meteorological station.*
Fig. 3 - Andamento in due anni e mezzo della temperatura media giornaliera, escursione termica e precipitazione nella stazione meteorologica di Arco.

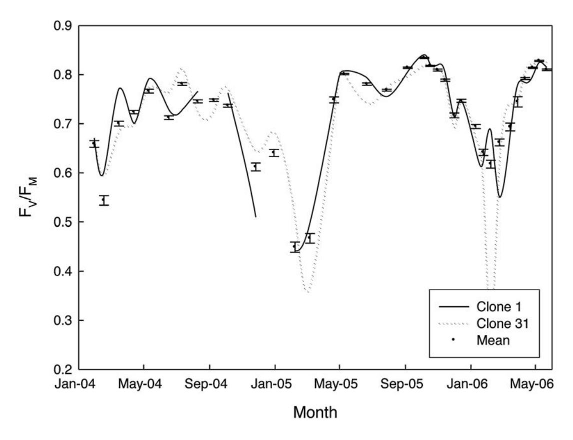

Fig. 4 - Seasonal course of F_V/F_M in cypress. Continuous and dotted lines represent the most different behaviors, respectively of clone 1 and 31. Dots with error bars show the mean values of all 99 clones and the standard errors.
Fig. 4 - Andamento stagionale di F_V/F_M nel cipresso. Le linee continue e tratteggiate si riferiscono ai cloni più diversi tra loro, rispettivamente il clone 1 e il 31. I tratti con le barre di errore mostrano il valore medio sui 99 cloni e gli errori standard.

used to correlate the F_V/F_M data and to estimate a threshold temperature for cypress photochemical activity.

Fig. 5 shows the trend of the Pearson correlation coefficient performances of micrometeorological parameters and the mean values of F_V/F_M of the cypress clones, with changes in temperature threshold for the calculation of cooling degrees from the maximum (A), medium (B) and minimum (C) air temperature. Most of the correlations between air-temperature based indices and the mean values of F_V/F_M proved to be highly significant ($p<0.001$; Tab. 2). Only the relationship between the 3-day running mean of maximum daily air temperature ($T_{max}RM3$) and F_V/F_M showed higher p value (0.038), but still significant ($p<0.05$). The highest correlation ($r = -0.852$; $p<0.001$) was found between (F_V/F_M) and 10-day cold-degree-

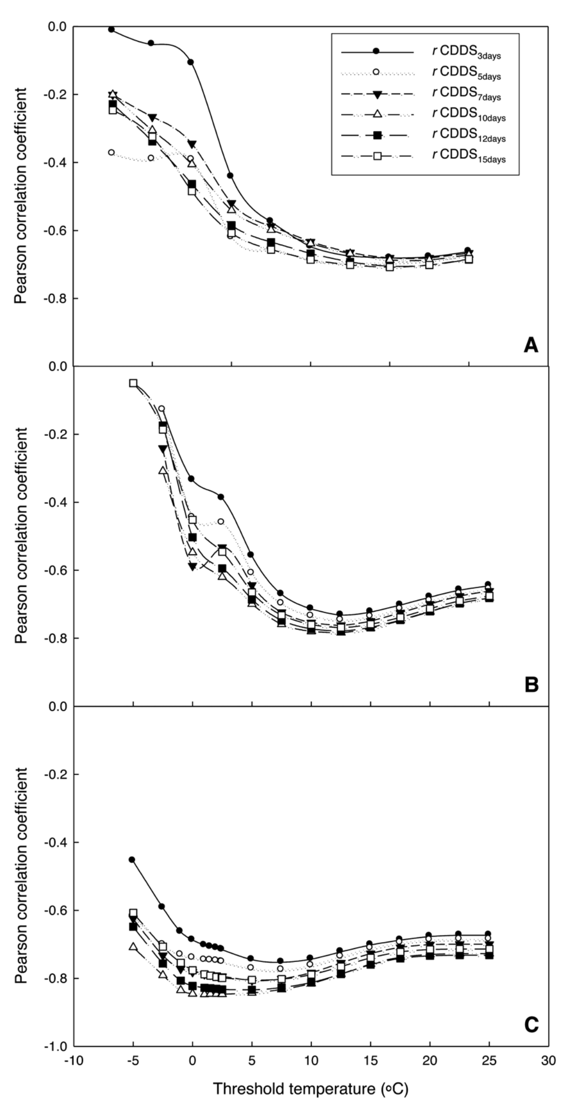

Fig. 5 - Correlation performances with changes in temperature threshold for the calculation of cooling degrees from the maximum (A), medium (B) and minimum (C) air temperature.
Fig. 5 - Risultati di correlazione con valori variabili delle soglie di temperatura per il calcolo dei gradi di freddo con le temperature massime (A), medie (B) e minime (C).

Parameter	Pearson coefficient	p	Parameter	Pearson coefficient	p
T_{min}	0.675	<0.001	$T_{min}CDDRS_7\ T_S$=2°C	-0.836	<0.001
T_{mean}	0.640	<0.001	**$T_{min}CDDRS_{10}\ T_S$=2°C**	**-0.852**	**<0.001**
T_{max}	0.587	<0.001	$T_{min}CDDRS_{12}\ T_S$=2°C	-0.824	<0.001
$T_{min}RM_3$	0.636	<0.001	$T_{min}CDDRS_{15}\ T_S$=2°C	-0.805	<0.001
$T_{min}RM_5$	0.670	<0.001	$T_{mean}\ CDDRS_3\ T_S$=12°C	-0.738	<0.001
$T_{min}RM_7$	0.686	<0.001	$T_{mean}\ CDDRS_5\ T_S$=12°C	-0.764	<0.001
$T_{min}RM_{10}$	0.736	<0.001	$T_{mean}\ CDDRS_7\ T_S$=12°C	-0.778	<0.001
$T_{mean}\ RM_3$	0.659	<0.001	$T_{mean}\ CDDRS_{10}\ T_S$=12°C	-0.788	<0.001
$T_{mean}\ RM_5$	0.670	<0.001	$T_{mean}\ CDDRS_{12}\ T_S$=12°C	-0.774	<0.001
$T_{mean}\ RM_7$	0.659	<0.001	$T_{mean}\ CDDRS_{15}\ T_S$=12°C	-0.773	<0.001
$T_{mean}\ RM_{10}$	0.679	<0.001	$T_{max}CDDRS_3\ T_S$=20°C	-0.619	<0.001
$T_{max}RM_3$	0.352	0.038	$T_{max}CDDRS_5\ T_S$=20°C	-0.660	<0.001
$T_{max}RM_5$	0.645	<0.001	$T_{max}CDDRS_7\ T_S$=20°C	-0.660	<0.001
$T_{max}RM_7$	0.634	<0.001	$T_{max}CDDRS_{10}\ T_S$=20°C	-0.689	<0.001
$T_{max}RM_{10}$	0.611	<0.001	$T_{max}CDDRS_{12}\ T_S$=20°C	-0.690	<0.001
$T_{min}CDDRS_3\ T_S$=2°C	-0.762	<0.001	$T_{max}CDDRS_{15}\ T_S$=20°C	-0.705	<0.001
$T_{min}CDDRS_5\ T_S$=2°C	-0.797	<0.001			

Tab. 2 - Pearson correlation coefficients (r) and significance level (p) of the correlations between the reported micrometeorological parameters and the mean values of F_V/F_M of the cypress clones. The best performing model (with minimum, mean, and maximum temperature) is highlighted in bold characters.

Tab. 2 - Coefficienti di correlazione di Pearson (r) e livello di significatività (p) delle correlazioni tra i parametri micrometeorologici riportati e i valori medi di F_V/F_M per i cloni di cipresso. Il modello migliore (con le temperature minime, medie e massime) è evidenziato in neretto.

day running sum of minimum air temperature (T_{min}CDDRS10) with a threshold value (TS) of 2 °C (Fig. 6). For the mean air temperature the strongest correlation was found at 12 °C.

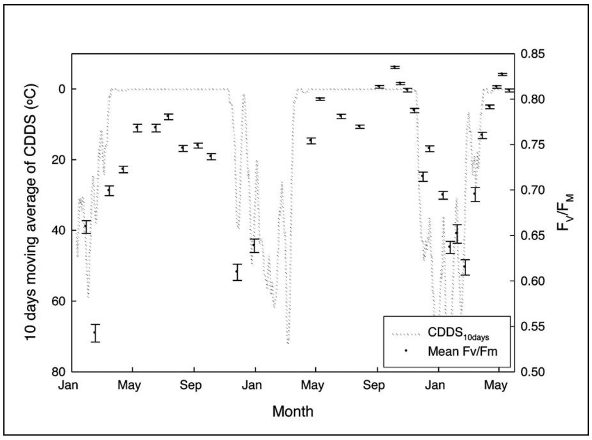

Fig. 6 - T_{min}CDDRS10 (dotted line - note the reversed y axis on the graph) and mean values and standard errors of F_V/F_M for the 99 clones (dots with error bars).

Fig. 6 - T_{min}CDDRS10 (linea punteggiata – asse y con scala inversa) a valori medi con errore standard dei valori di F_V/F_M misurati per i 99 cloni (punti con barre d'errore).

DISCUSSION

Night frosts in the early autumn resulted in minor and temporary declines in F_V/F_M measured in cypress clones grown in Trentino. However, by early November a permanent decrease in F_V/F_M occurred, correlated to a decline in mean air temperatures. This pattern was consistent for all clones. A similar reduction in photosynthetic capacity during winter has been observed in many other conifer species, including white spruce (Binder and Fielder, 1996), Norway spruce (Lundmark *et al.*, 1988; Bolhàr-Nordenkampf and Öquist, 1993; Adams and Perkins, 1993; Westin *et al.*, 1995; Weng *et al.*, 2005), red spruce (Lawson *et al.*, 2000), black spruce (Gaumont-Guay *et al.*, 2003), Douglas fir (Rose and Haase, 2002; Nippert *et al.*, 2004), western redcedar (Weger *et al.*, 1993; Nippert *et al.*, 2004), and Scots pine (Porcar-Castell *et al.*, 2008; Robakowski and Wyka, 2009), Engelmann spruce (Nippert *et al.*, 2004), grand fir (Nippert *et al.*, 2004), ponderosa pine (Nippert *et al.*, 2004) and lodgepole pine (Nippert *et al.*, 2004).

Previous meteorological data were necessary to predict current photosynthetic capacity of cypress in the wintertime, the maximum correlation being observed with the cold-degree-days sum of minimum air temperature below 2 °C. Schaberg *et al.* (1995) reported the highest correlation between winter net photosynthetis in red spruce and maximum daily temperature occurring 4–6 days before measurement, and Lawson *et al.* (2000) found the patterns of winter F_V/F_M measurements of the same species to be significantly correlated with current air temperatures over the preceding 72 h. Tanja *et al.* (2003) found that photosynthetic rates estimated from eddy flux data were best correlated with a 5-day running mean temperature in boreal forests of Finland, Sweden and central Siberia (Russia). Medlyn *et al.* (2002) reported the strongest correlation between the maximum rate of carboxylation and the potential rate of electron transport in maritime pine to mean daily minimum temperature over the previous 30 days. Nippert *et al.* (2004) found similar delays in the response of photosynthetic capacity to changing ambient temperature, but the nature of the relationship and length of the delay differed among several conifer species in northern Idaho (USA). Mechanisms controlling photosynthetic machinery during winter are slow to damage or repair and vary among species. Clones differed in susceptibility to winter photoinhibition, which was probably related to their specific stress-tolerance mechanisms and different light requirements (Baldi *et al.*, 2012). Winter photoinhibition in cypress was not associated with permanent damage to photosynthetic machinery, because recovery of F_V/F_M was observed with increasing air temperature in all clones. The absence of persistent winter damage was further verified during consecutive growing seasons. Presumably the long-lived "needles" of this species (functioning for several years) experience recurrent slight winter photoinhibition, triggering acclimation mechanisms, which makes their spring recovery possible each year. It may be hypothesized that the low-temperature photoinhibition observed in the present study played a photoprotective role (Somersalo and Krause, 1990; Robakowski, 2005).

In situ experiments would have probably revealed less between-clone variation as evidenced by the present common garden experiment, because of environmental determinism due to clone-site interaction. Indeed, while differences in phenological traits observed *in situ* reflect both environmental and genetic variations, common garden experiments are related to genetic variation only. Higher exposure to air of scattered trees in the field would make them experience critically low temperatures. Nevertheless, the observed intraclonal variability of the studied functional trait indicates substantial genetic diversity within genotypes, which could potentially facilitate rapid adaptation to changing environmental conditions. Local adaptations, however, influence phenotypic variability *in situ*.

Winter photosynthetic rates were not measured directly in this study. However, the relatively smaller reduction in F_V/F_M in several clones in winter compared with the others one may be explained by a relatively higher winter photosynthetic capacity, which provides a sufficient sink for absorbed solar energy, thereby preventing photo-oxidative stress. Throughout the winter, from November until the beginning of April, photochemical activity was relatively low and stable with F_V/F_M values ranging from values below 0.3 to above 0.6, depending on the clone. Light-demanding pioneer species are generally less susceptible to photoinhibition than shade-tolerant climax species (Krause *et al.*, 2001). In cypress, susceptibility to photoinhibition in the winter and early spring depended on prevailing air temperature and genotype more than light (Baldi *et al.*, 2012), occurring at temperatures well above the lethal minimum temperature (Öquist and Huner, 2003).

In winter, photosynthesis can eventually be constrained by photoinhibition (Öquist and Huner, 2003), desiccation due to extracellular freezing (Neuner and Pramsohler, 2006), xylem blockage due to freezing of the stem, roots or soils (Pittermann *et al.*, 2010), and inhibition of the dark reactions of photosynthesis (Adams and Perkins, 2004), acting independently or in combination as a correlated set of responses through the acclimation process (Öquist and Huner, 2003). Each would have different controls and different lag times, and each would have different effects on F_V/F_M. Therefore empirical descriptions of the temperature–response curves may offer clues as to which are most important in a given system.

Although the weather during winter was occasionally mild period, recovery of the photochemical activity to high values was not observed in any of the clones. Because of the time interval between measurements during winter, it is possible that the actual variation in F_V/F_M was larger than reported here. Extreme events such as frost, with temperature lower than -10 °C, were recorded at the nursery, but consecutive frost days were infrequent and the effect of these episodes on the photochemical activity of cypress was not completely investigated. Most conifers tolerate much colder temperatures than the night-time minima reported here (Strand and Lundmark, 1987), and it is unlikely that such episodes act as the major control agent in acclimated vegetation (Strand *et al.*, 2002), and in orchard conditions. However, if dehydration due to extracellular freezing were the mechanism of loss, then T_{min} would better predict F_V/F_M than T_{max}, because the freezing would be unaffected by light and would proceed furthest at the lowest temperatures reached. Moreover, because the initial cytoplasmic freezing would be rapid and the recovery from freezing damage would probably be slow (Schwarz *et al.*, 1997), we might be unable to distinguish between photoinhibition and extracellular freezing as potential mechanisms leading to the loss of photosynthetic capacity (Nippert *et al.*, 2004).

The spring recovery in F_V/F_M values was rapid in all clones and occurred when air temperatures increased in April-May, which is in accordance with recovery studies in Scots pine (Ottander and Öquist 1991) and Norway spruce (Westin *et al.*, 1995). In springtime, the reversal of photoinhibition may be slow at low temperatures (Lamontagne *et al.*, 1998), leading to long lag times, when bright light and low temperature combine their effects during daytime (presumably in association with T_{max}).

Trees cannot sustain water losses due to transpiration if their stems or roots (or soils) are frozen. Therefore, xylem (and soil) freezing may be a major control over winter photosynthetic processes (Schwarz *et al.*, 1997; Jarvis and Linder, 2000). Although we cannot address this question directly with our data, and a direct correlation between xylem freezing and changes in fluorescence has yet to be documented, the cold-degree-days sum of minimum air temperature below 2 °C represented the best correlation with F_V/F_M. The rate of dark reactions of photosynthesis are strongly dependent on temperature, especially as temperature approaches 0 °C (Strand *et al.*, 2002; Gaumont-Guay *et al.*, 2003), and control photosynthetic rates during the wintertime. Dark-acclimated chlorophyll fluorescence measures a photochemical trait, though this effect could still be detected if induced by feedback inhibition (Öquist and Huner, 2003). While all these four mechanisms have the potential to limit wintertime photosynthesis, our results suggest that photoinhibition, and perhaps xylem freezing, are the most probable candidates.

The extent to which cypress clones are able to adapt to environmental changes will depend on the level and distribution of genetic variation between genotypes and phenotypic plasticity, other than on seed dispersal and establishment rate. Our results show that, despite gene flow between clones due to their proximity, genotypes may nonetheless display genetic differentiation for functional traits due to diversifying selection along temperature gradients. As large within-genotype differentiation are possible for functional traits, due to selection occurring during past natural warming, we suspect that substantial adaptive evolutionary changes are likely to occur in response to current climate change. The migration of cypress will be facilitated by the extensive genetic diversity within populations and phenotypic plasticity. However, the lack of systematic, long-term monitoring of species distribution, morphology, physiology and other critical responses of Mediterranean species is hampering our ability to predict the impact of climate change on biodiversity and productivity in these changing landscapes.

The duration of seasonal growth influences the annual estimates of conifer productivity (Bergh *et al.*, 1998). To reflect annual production accurately, a more detailed understanding of seasonal growth and temperature response is needed (Leverenz and Öquist, 1987; Medlyn *et al.*, 2002). Leverenz and Öquist (1987) reported seasonal differences in the response of quantum yield to varying temperature that make it difficult to define maximum quantum yields based on temperature alone. Predictions may be improved with accounts of cold acclimation and winter differences in photoinhibition (Gaumont-Guay *et al.*, 2003), direct reduction in photosynthesis (Linder and Flower-Ellis, 1992; McMurtrie *et al.*, 1994; Bergh *et al.*, 1998), variation in soil temperatures (Bergh and Linder, 1999), and seasonal differences in photosynthetic recovery (Linder and Flower-Ellis, 1992; McMurtrie *et al.*, 1994; Bergh *et al.*, 1998; Lundmark *et al.*, 1998; Medlyn *et al.*, 2002; Zunzunegui *et al.*, 2011). The model describing the best correlation between environmental parameters and F_V/F_M illustrates the complexity of this system in a Mediterranean environment even during the wintertime. However, fluorescence measurements provide a quick and reliable method to describe this complication for future analyses and model predictions (Nippert *et al.*, 2004), particularly in screening studies in nursery conditions.

This study at the nursery scale aimed at providing insight into the specific factors determining the patterns of species distribution associated with air temperature gradients. We observed similar trends for 99 clones from distant sites, indicating that local drivers of functional leaf traits may be consistent at the regional scale. Nevertheless, cypress is distributed across all the latitudinal range of the Mediterranean Basin and can display substantial population differences, as observed in other conifers (Zhang and Marshall, 1994; 1995).

As global warming impacts are predicted to increase in the next decades, it is likely that the cypress will expand its range in the Alps. Under this perspective, a much wider spread of cypress due to human plantations is also expected and the availability of adapted genotypes will be a key issue in the future use of cypress as ornamental tree at the Northern margin of its range.

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How a traditional agricultural protection structure acts in conditioning the internal microclimate: a statistical analytical approach to Giardino Pantesco (Pantelleria Island - Italy)

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Abstract: *This work aims to analyze the traditional agricultural technique of the Giardino Pantesco, typical of the isle of Pantelleria (Sicily, Italy). With this particular technique a single citrus tree is encircled by a drywall of volcanic rocks allowing it to grow even in the island's unfavorable meteorological conditions. This technique has been analyzed by placing one instrumental array inside the Giardino and one outside and by measuring different environmental variables. The aim of the work is therefore to understand what is the drywall's effect on the tree by analyzing the time series produced by the sensors, using cross-correlation techniques and reducing the series' autocorrelation, so that the time series are not biased by variables' memory. The outcome of the analysis shows that the wall acts in different ways depending on the variable in consideration and tends to buffer environmental extremes which can potentially harm the citrus tree.*

Keywords: *traditional agricultural knowledge, time series analysis, Sicily.*

Riassunto: *Questo lavoro punta ad analizzare la tecnica agricola tradizionale del Giardino Pantesco, tipico dell'isola di Pantelleria (Sicilia, Italia). Questa particolare tecnica prevede di circondare un singolo albero di agrumi con un muro a secco di roccia vulcanica, permettendogli di crescere anche nelle condizioni meteorologiche sfavorevoli dell'isola. Tale tecnica è stata analizzata installando un array strumentale all'interno del Giardino e uno all'esterno e misurando diverse variabili ambientali. Lo scopo del lavoro è pertanto comprendere quale sia l'effetto del muro a secco sull'albero analizzando le serie storiche prodotte dai sensori, usando tecniche di cross-correlazione e riducendo l'autocorrelazione delle serie in modo tale che non siano influenzate dalla memoria delle variabili. Il risultato dell'analisi mostra come il muro agisca in modi differenti a seconda della variabile presa in esame e come tenda a tamponare estremi ambientali che potrebbero potenzialmente danneggiare la pianta.*

Parole chiave: *conoscenza agricola tradizionale, analisi di serie storiche, Sicilia.*

1. INTRODUCTION

Fruit trees were first grown within gardens as stated for example by the Bible (Genesis 2,8 -14, the Garden of Eden) and by the Homer's Odissey (VII, 113-130) probably due to the idea that a close, protective environment was needed to grow such demanding, long term crops. Several years of farming dedication after plantation were needed for trees to be harvested, and the high nutritional value of fruits complemented with aesthetics and symbolic appreciation. Also difficult environments were colonized by fruit trees, and their cultivation became soon, many millenniums ago, a smart farming exercise, in which control of tree growth combined with protection from high winds, excess of evapotranspiration and predators. One of the most ancient representations is the Sumerian "life garden", painted in a tablet in the III millennium B.C. (Fig. 1.1). Such a structure, in which a single tree is grown within a protection wall, is still largely present in the Island of Pantelleria (Sicily, Italy) where takes the name of giardino Pantesco (Fig. 1.2 and 1.3).

This garden is an important element of the traditional landscape and of local subsistence agriculture of the isle. In it, a single citrus tree, usually orange, is encircled in a drywall of local volcanic rocks almost as high as the tree itself, to create an internal microenvironment enabling tree growth even in an unfavorable climate such as the one of the isle (extremely low rainfalls and high wind speeds throughout the year) (Drago, 2005).

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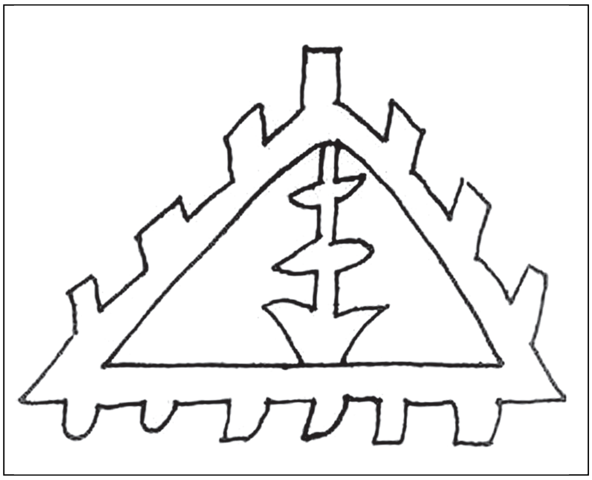

Fig. 1.1 - The Garden of Life: The enclosed Life Garden represented in a Sumerian tablet. (from Barbera. G, 2007).
Fig. 1.1 - Il Giardino della Vita: Il Giardino della Vita, circondato da un muro, come rappresentato in una tavoletta Sumera. (da Barbera G., 2007).

Fig. 1.2 - The Pantesco Garden: The garden in which this study has been carried on.
Fig. 1.2 - Il Giardino Pantesco: Il giardino nel quale è stato condotto il presente studio.

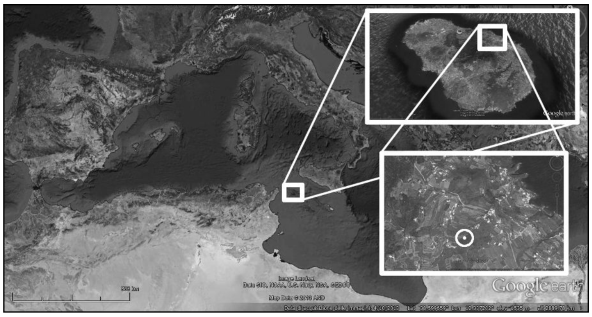

Fig. 1.3 - Location of the Garden: synthetic map of the Mediterranean area highlighting the location of the Island of Pantelleria and of the Pantesco Garden inside the island itself.
Fig. 1.3 - Posizione del Giardino: mappa sintetica dell'area Mediterranea che evidenzia la posizione dell'Isola di Pantelleria e del Giardino Pantesco all'interno dell'isola stessa.

The analysis reported here aimed to investigate, by using a numerical analysis, the physical modifications occurring within the garden, and highlight differences and analogies between the in-wall and the out-wall environments. Data on wind speed, air relative humidity and temperature were treated to reduce autocorrelation and to obtain stationary time series enabling a comparison unbiased by variables memory.

2. MATERIALS AND METHODS

Two masts were installed inside and outside the garden, respectively at a height of about one half the garden's wall (i.e.: 1.5 meters considering an average wall height of 2.7 meters) and at 3.8 meters, and were supporting a large instrumental array of meteorological and soil sensors. For this current analysis, only a subset of the whole instrumentation has been considered, and includes a hygrometer (measuring the air relative humidity, RH), a copper-constantan thermocouple (air temperature) and a cup anemometer (wind speed and direction).

Two 10-days subsets of data (fall period: 15 November- 25 November 2008, spring period: 28 March 2009- 08 April 2009), when hourly time series had enough numerosity to be statistically relevant (Dagum, 2002), were taken for the comparison.

To assess the representativeness of those two periods to the typical Pantelleria's meteorological trends, data were compared with long term-trends coming from the "Atlante Climatico" of the Italian Air Force (CNMCA, 2009). The "Atlante" contains data collected between 1971-2000 from airport-hosted meteorological stations across all Italy (including the Island of Pantelleria). This long term data support our short-term observations during the sampling periods and therefore their climatic representativeness.

Since the wall is not completely closed (it encircles the tree, but has no topping) major environmental forcing and trends are likely to act both on the inside and on the outside of the garden's environment. Therefore, autocorrelation in the variables may produce similar trends and mask differences.

The procedure tested in this paper aims to reduce or eliminate such effects generating less autocorrelated and more stationary data for comparison.

To do this, the following approach is employed:

1. Preliminary analysis of the variables via bivariate time-plots (internal and external variable plotted simultaneously) and data pre-processing.
2. Autocorrelation and stationarity analysis of the single variables via:
    a. Lagged scatterplots

    b. Autocorrelation function
    c. Stationarity test and runs test
    d. Spectral analysis via fast Fourier transform
3. If the variable shows high autocorrelation in step 2, this effect is reduced via:
    a. Outlier removal
    b. Differencing
    c. De-trending
4. The effect of step 3 is analyzed as per step 2 and if necessary step 3 is repeated
5. Comparison of internal-external stabilized variables via:
    a. Bivariate time plots
    b. Cross-correlograms

Some explanations are hereafter given to better detail the statistical approach sketched in the previous points (1-5): in the first step (point 1) the internal and external variables are plotted against time to create a preliminary representation of their trends. In this phase a couple of pre-processing steps are applied in case of missing data or data signaling instrumental error. In the former case, since the gaps are very short (a maximum of 2 hours) they are filled with the means of the neighboring observations. In the latter case the anomalous data are removed and gap-filling proceeds in the same way. After the preliminary step the autocorrelation of the variable is then analyzed (point 2): with the usage of lagged scatterplots (2a) the variable is plotted against a lagged version of itself. If a time series of length N is composed of x_i observations, where i=1,…,N, the lagged scatterplot for lag k is then a scatterplot of the last N-k observations against the first T-k observations. Each scatterplot is fitted with a least square line in order to evaluate the effective "randomness" of the scatterplot, linearity and strength of relationship between current and past values. The correlation coefficient of the fitted line can be compared with a critical level of correlation required to reject the null hypothesis that the sample comes from a population with zero correlation at the indicated time lag.

Following Chatfield (2004): if sample size is sufficiently large and the time series from which is taken is completely random, the lagged-correlation coefficient is normally distributed with mean 0 and variance 1/N. Therefore the critical level of correlation for 95% significance is equal to $0\pm2/\sqrt{N}$ Results of the lagged scatterplot can be summed up by the autocorrelation function of the variable. Essentially the autocorrelation value for variable x at lag k can be computed as (Box *et al.*, 1994):

$$r_k = c_k/c_0 \qquad (1)$$

where c represents the autocovariance function computed respectively at lag k and at lag 0. The autocovariance function at lag k can be written as:

$$c_k = \frac{1}{N}\sum_{t=1}^{N-k}(x_t-\bar{x})(x_{(t+k)}-\bar{x}) \qquad (2)$$

where N is the total number of observations, and $\bar{x}$ represents the sample mean.

The autocorrelation function (2b), then, is essentially the correlation coefficient (computed also by the lagged scatterplots) at each lag k. Both methods are employed because they have an important graphical difference: while the autocorrelation resumes in a single graph a lot of time lags, the lagged scatterplot employs a single figure for each lag, but by showing explicitly the observations allows for recognition of eventual outliers. Lagged scatterplots are reported here to propose to the reader an integrated employable framework, and are used as an exploratory technique, but will not be shown for every variable in the article unless when needed for highlighting particularly interesting situations. The autocorrelation function and the lagged scatterplots can be side-kicked by the runs test (2c). The test is based on the number of runs of consecutive values above or below the mean of x. Too few runs indicate a tendency for high and low values to cluster. Too many runs indicate a tendency for high and low values to alternate. The test statistic approximates normal distribution when the null hypothesis (i.e.: the values of the analyzed variable come in random order) is true and is calculated as the difference between the number of runs and its means, divided by its standard deviation. The above mentioned analyses were carried out using MATLAB (version 2009a).

Along with the aforementioned analysis, series are tested with the Kwiatkowski *et al.* (1992) test for stationarity (2c, KPSS test from now on). Basically the test considers the analyzed time series y_t as decomposable into the sum of a deterministic trend, a random walk and a stationary error:

$$y_t = \xi t + r_t + \varepsilon_t \qquad (3)$$

In equation (3) r_t is a random walk:

$$r_t = r_{t-1} + u_t \qquad (4)$$

Where the u_t are identically and independent distributed random variables with mean 0 and variance σ^2_u. The initial value r_0 is treated as fixed and serving as an intercept. The stationarity hypothesis of the test is $\sigma^2_u=0$ and stands for stationarity of the time series y_t around the trend ξ. The test is also able to determine whether the series is stationary around the level r_0 when $\xi=0$. The test also considers an allowance for error autocorrelation (for further details please refer to Kwiatkowski *et al.*, 1992). For all of the aforementioned tests the significance level was set at $p <= 0.05$

Additional useful insights on the variables' behavior can stem from their spectra (2d). Details about the Fourier transform and its application to time series analysis are reported in Bloomfield (1976), Cadzow (1973), Howell (2011) and Warner (1998). What is important to know here is that a relationship between the spectrum of a time series and its variance exists. More precisely, the spectral power (as the square of the signal power) represents the autocorrelation function in the frequency domain, allowing the understanding of how the variance of the time series contributes to different frequencies (and, therefore, periods).

The spectral analysis is performed as the last step since the previous tests and graphical representations allow for the determination of possible trends in the series which can "clutter" the spectral plot and need to be removed. De-trending (3c) is obtained via iterative fitting of polynomial curves to the series and removal of the curve best fitting the identified trend from the actual data. Otherwise, differencing (3b) at different time lags can also contribute to stabilize and de-trend the series (this method has proven effective and has been extensively used by the authors). Prior to operating spectral analysis, given our aim in confronting the series, the data are divided by their standard deviation in order to normalize the variance and make the resulting spectra comparable.

In practice the raw periodograms of the variables are used and are evaluated in two steps:

- Removing the mean from the data.
- Plotting the first half of the squared fast Fourier transform of the data against frequency.

During the whole process described in these pages, the removal of outliers (3a) is utilized as less as possible and only to remove observations clearly outside of the instrumental range or coming from instrumental failures: removing data in such short-spanned environmental time series poses the threat to remove important physical phenomena which are, instead, very effective on plant life. This is also why no mobile average approach is used by the authors.

To test the effectiveness of the methods described in step 3, the whole step 2 and its sub-steps need to be employed again.

In the end (point 5), the stationary time series of the corresponding internal and external environmental variable (i.e.: internal wind speed and external wind speed) are compared using different methods: bivariate time plots are employed to give a temporal representation of the stationary series, while cross-correlation functions allow to assess the relationship and the strength of the relationship between the two variables.

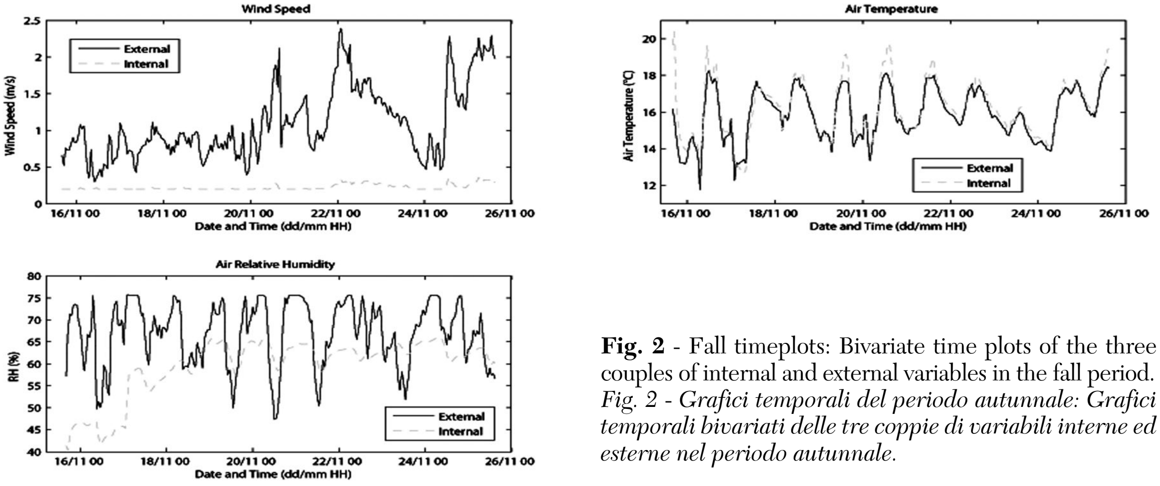

Fig. 2 - Fall timeplots: Bivariate time plots of the three couples of internal and external variables in the fall period.
Fig. 2 - Grafici temporali del periodo autunnale: Grafici temporali bivariati delle tre coppie di variabili interne ed esterne nel periodo autunnale.

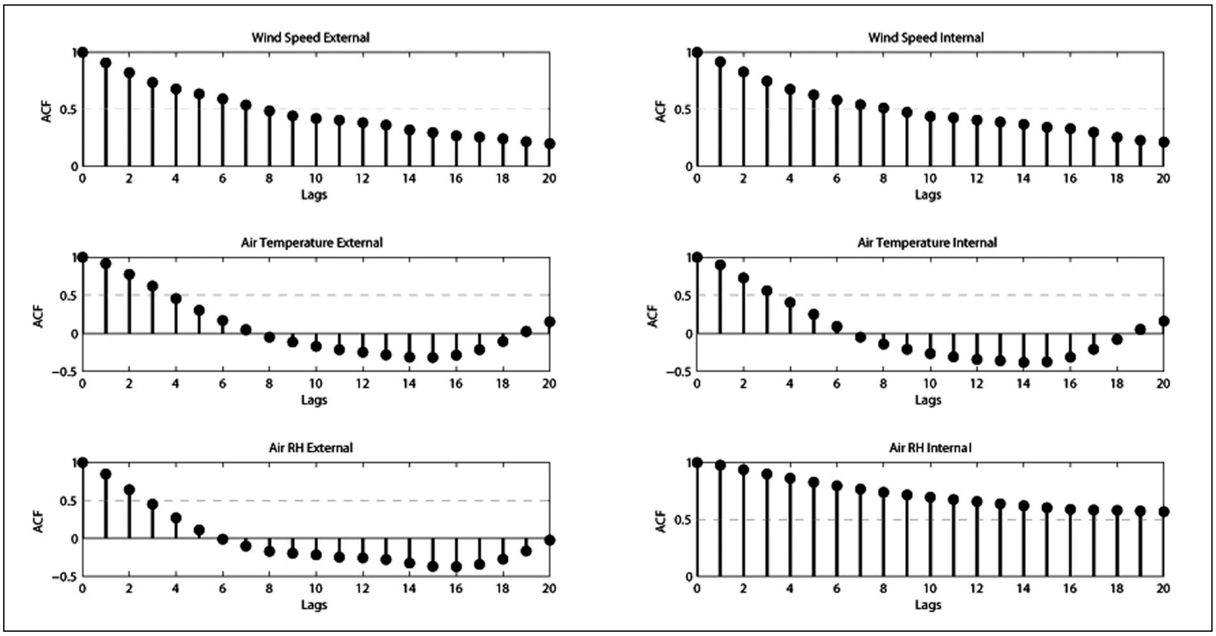

Fig. 3 - Fall autocorrelations: Autocorrelation functions for the three couples of internal and external variables in the fall period. The dashed grey line highlights the 0.5 autocorrelation level.
Fig. 3 - Autocorrelazioni autunnali: Funzioni di autocorrelazione per le tre coppie di variabili interne ed esterne nel periodo autunnale. La linea grigia tratteggiata evidenzia il livello 0.5 di correlazione.

3. RESULTS AND DISCUSSION

3.1 Fall Period

The simple analysis of the time plots gives out some important details: internal wind speed values are in fact much lower than the external ones. The internal wind speed trend is not clearly visible in this graphical representation, but it follows the external one. Also, the internal air temperature shows values generally slightly higher than the external one, but the time trends of the two variables are very similar. Internal air relative humidity, instead, does not closely follow the external humidity trend. The following figure (Fig. 2) shows bivariate time plots for the fall period of the three variables recorded externally (black solid line) and internally (dashed line).

Wind speed (both internal and external) shows high autocorrelation from lag 0 to lag 8, while air temperature (both internal and external) is autocorrelated from lag 0 to lag 4. More interestingly, the humidity, which showed different trending in the bivariate time plot, also evidences different autocorrelation plots between external and internal locations. Moreover while the external RH is significantly autocorrelated from lag 0 to lag 3, the internal RH exhibits significant autocorrelation even at the twentieth lag. The autocorrelation functions for the three couples of variables are reported in the following figure (Fig. 3).

The results of the tests (Tab. 1) show that all variables have not independent values, and these results can be expected due to the high autocorrelation (highly autocorrelated variables have "memory", therefore their values tend to be similar and correlated with the preceding ones and therefore generate many runs above or below the mean when a runs test is made). External wind speed, internal wind speed and internal RH show also non-stationarity when the KPSS test is applied, hinting that some trend removal procedures may be due to make these variables stationary.

For wind speed, the procedure found to be the most effective is the differencing of the original variables at lag 1. The differencing of these two variables effectively removed autocorrelation at every time lag and shows that the two variables still maintain a cross correlation at lag 0 (Fig. 4), indicating that the internal and external environment are influenced by the same forcing. The effect of the garden's wall in this case is only to buffer wind speed.

From the bivariate time plots of the differenced variables (Fig. 4, top plot on the left) it is clear that the internal wind speed shows lower values than the external variable, and such values are "contained" between the external wind speed's variability. The internal wind speed's after the differentiation appears very smooth because it has many constant values and the differentiation transforms them to 0. Since we are

	External Wind Speed	Internal Wind Speed	External Air Temperature	Internal Air Temperature	External Air Relative Humidity	Internal Air Relative Humidity
Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity						
Statistic	2.554	2.472	0.398	0.218	0.043	3.40
p-value	<0.01	<0.01	0.08	>0.1	>0.1	<0.01
Result	H0 rejected	H0 rejected	H0 not rejected	H0 not rejected	H0 not rejected	H0 not rejected
Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity						
Statistic	0.141	0.192	0.119	0.073	0.042	1.0
p-value	0.059	0.019	0.1	>0.1	>0.1	<0.01
Result	H0 rejected	H0 rejected	H0 not rejected	H0 not rejected	H0 not rejected	H0 rejected
Runs Test						
Statistic	-12.597	-14.073	-12.612	-12.863	-10.104	-14.14
p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Result	H0 rejected	H0 rejected	H0 rejected	H0 rejected	H0 rejected	H0 rejected

Tab. 1 - Statistics of fall period.
Tab. 1 - Statistiche del periodo autunnale.

interested in trend analysis, having zero values or above zero constant values make no difference. This, obviously, reduces the variable's dynamics but still allows to notice that the internal wind speed tends to follow the external one also referring to the non-differenced time series. Since the transformation is so effective, there is no need to explore lagged scatterplots for this couple of variables. The effectiveness of the transformation is tested by repeating the KPSS and the runs test (Tab. 2).

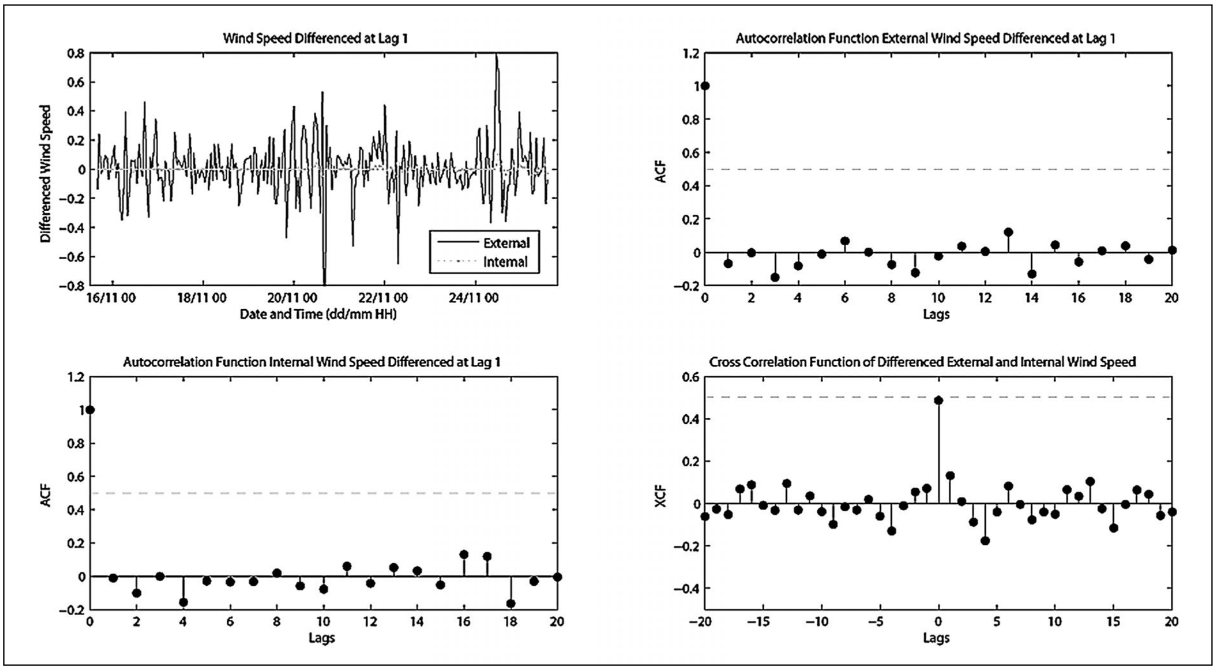

Fig. 4 - Plots of differenced wind speed (fall): From left to right and top to bottom: bivariate time plot of internal and external wind speed after differencing at lag 1, autocorrelation plot of external wind speed after differencing at lag 1, autocorrelation plot of internal wind speed after differencing at lag 1 and cross correlation plot of external and internal wind speed after differencing at lag 1.
Fig. 4 - Grafici per la velocità del vento differenziata (autunno): Da sinistra a destra e dall'alto in basso: grafico temporale bivariato della velocità del vento interna ed esterna dopo la differenziazione a lag 1, grafico di autocorrelazione della velocità esterna del vento dopo la differenziazione a lag 1, grafico di autocorrelazione della velocità del vento interna dopo la differenziazione a lag 1 e grafico di cross correlazione della velocità esterna ed interna del vento dopo la differenziazione a lag 1.

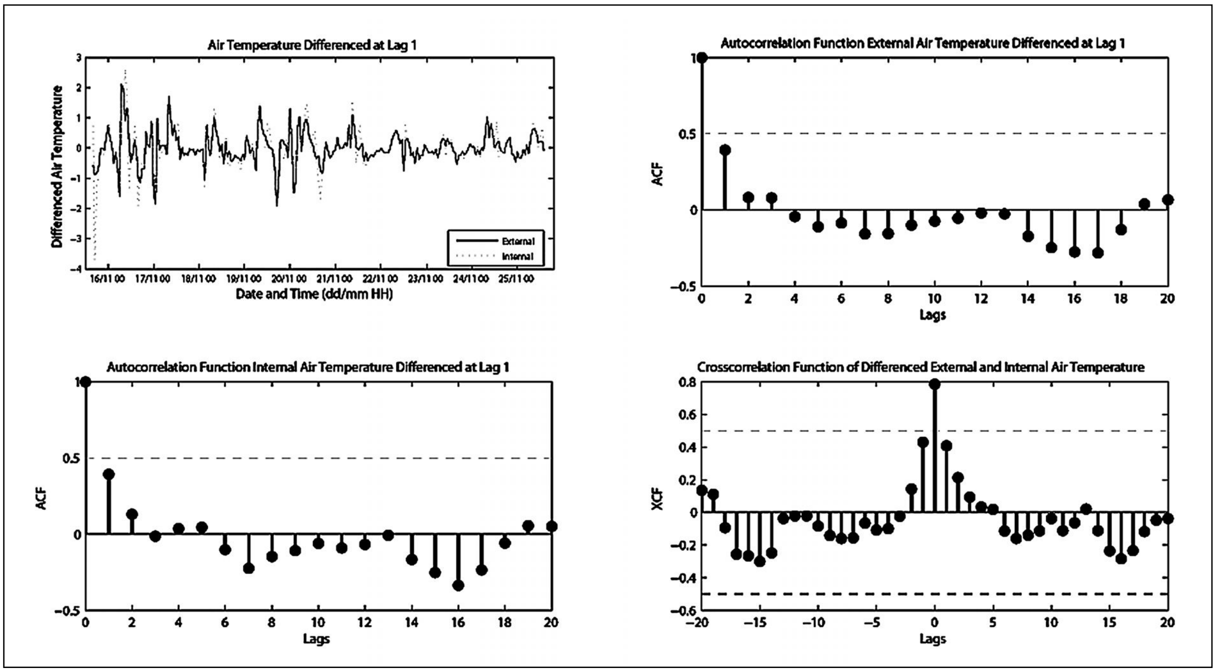

Fig. 5 - Plots of differenced air temperature (fall): From left to right and top to bottom: bivariate time plot of internal and external air temperature after differencing at lag 1, autocorrelation plot of external air temperature after differencing at lag 1, autocorrelation plot of internal air temperature after differencing at lag 1 and cross correlation plot of external and internal air temperature after differencing at lag 1.
Fig. 5 - Grafici della temperature dell'aria differenziata (autunno): Da sinistra a destra e dall'alto in basso: grafico temporale bivariato della temperatura dell'aria interna ed esterna dopo la differenziazione a lag 1, grafico di autocorrelazione della temperatura dell'aria esterna dopo la differenziazione a lag 1, grafico di autocorrelazione della temperatura dell'aria interna dopo la differenziazione a lag 1 e grafico di cross correlazione della temperatura dell'aria esterna ed interna dopo la differenziazione a lag 1.

Even for external and internal temperature, the differentiation at lag 1 results the most viable transformation in terms of reduction of variable memory and stability around a level or trend, while still maintaining a physical significance of the analyzed data. No strong "memory" exists after lag 0 (Fig. 5) but, still, the two variables are highly cross-correlated at lag 0. This means that the garden's wall does not decouple the thermal regime: the temperature on the inside is slightly higher, but follows the same forcing as the temperature outside.

The KPSS test confirms the stability over time of the analyzed variables. The runs-test, instead, seems to indicate that the sequence cannot be ascribed to a random sequence of value, therefore supporting an interdep endence of the variables' values (Tab. 3). The

	Differenced External Wind Speed	**Differenced Internal Wind Speed**
Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity		
Statistic	0.262	0.260
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity		
Statistic	0.205	0.0184
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Runs Test		
Statistic	0.594	0.165
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected

Tab. 2 - Statistics of differenced wind speed (Fall).
Tab. 2 - Statistiche per la velocità del vento differenziata (Autunno).

basic form of the runs test is based on the number of runs of consecutive values above or below the sample mean: too few runs indicate the clustering of high values with high values and low values with low values (potential trends), while too many runs indicate the tendency of high and low values to alternate (potential spikes).

Since no particular trends could be evidenced and removed via polynomial fitting or outlier removal via examination of lagged scatterplots (not reported), and no other transformation performed as good as differencing at lag 1, the runs test was re-utilized with the "UD" (up and down) parameter: this performs a test for the number of runs up or down and also tests the hypothesis that the values in the selected variable come in a random order. This version of the test looks for sequences of numbers: an "up" run is a sequence of numbers each of which is succeeded by a larger number, while a "down" run is a sequence where each number is succeeded by a smaller one. Too few runs are an indication of a trend, while too many runs indicate a tendency to oscillate. Used in this fashion, the test agrees both with the autocorrelation functions and the KPSS tests (Tab. 4).

The stabilization of air relative humidity has required a multiple application of the Box and Jenkins regular differencing (Box and Jenkins, 1994): the differencing at lag 1 has been applied twice in the case of external air relative humidity and thrice in the case of the internal air relative humidity. The effects of differencing are shown in the following figure (Fig. 6): the buffer effect of the wall is again visible. The values of internal air RH are "contained" in the external RH values. The garden wall, therefore, acts in buffering the fluctuations of relative humidity thus shielding the plant from humidity extremes. The cross-correlation function shows that at lag 0 there's no correlation: the wall decouples the variables creating an internal regime that's different from the external one.

The auto correlation functions and the KPSS test agree well (Tab. 5), while the standard formulation of the runs-test individuates a non-independence between values (Tab. 5).

The 'UD' version, instead, shows the effectiveness of differentiation in stabilizing the variables (Tab. 6).

The difficulty of interpreting the runs-test on the internal and external RH lies in the fact that there is an alternation of high and low values, which seems to be characteristic of the relative humidity probably due to some day-night cycles (as for temperature, see Fig. 2).

Globally, during the fall period, the presence of the wall has caused different effects on the different variables.

For wind speed, the wall buffered the strength of the wind within the garden without significantly altering its trend, indicating a non-decoupling of the internal and the external variable. This effect allows the tree to grow and produce in a mitigated wind speed condition.

For air temperature, the wall did not show any buffering effect: the internal climate was slightly

	Differenced External Air Temperature	**Differenced Internal Air Temperature**
	Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity	
Statistic	0.033	0.084
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
	Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity	
Statistic	0.021	0.030
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
	Runs Test	
Statistic	-6.776	-5.163
p-value	<0.01	<0.01
Result	H0 rejected	H0 rejected

Tab. 3 - Statistics for differenced air temperature (Fall).
Tab. 3 - Statistiche per la temperatura dell'aria differenziata (Autunno).

	Differenced External Air Temperature	**Differenced Internal Air Temperature**
	Runs Test 'UD'	
Statistic	-1.582	-1.054
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected

Tab. 4 - "Up-down" test statistics for differenced air temperature (Fall).
Tab. 4 - Statistiche del test "up-down" per la temperatura dell'aria differenziata (Autunno).

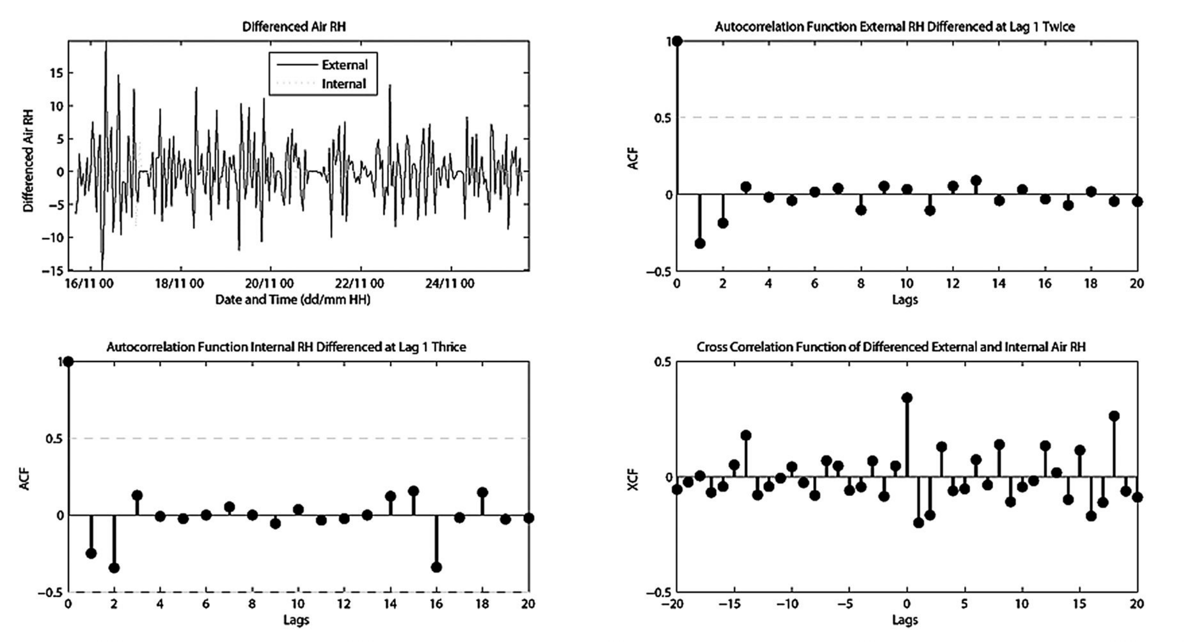

Fig. 6 - Plots for differenced air RH (fall): From left to right and top to bottom: bivariate time plot of internal and external air RH after differencing at lag 1 twice (for external RH) and thrice (for internal RH), autocorrelation plot of external air RH after differencing at lag 1 twice, autocorrelation plot of internal air RH after differencing at lag 1 thrice and cross correlation plot of external and internal RH after differencing.

Fig. 6 - Grafici per l'umidità relativa dell'aria differenziata (autunno): Da sinistra a destra e dall'alto in basso: grafico temporale bivariato dell'umidità relativa dell'aria interna ed esterna dopo una doppia differenziazione a lag 1 (per l'umidità esterna), e una tripla differenziazione (per l'umidità interna), grafico di autocorrelazione dell'umidità dell'aria esterne dopo una doppia differenziazione a lag 1, grafico di autocorrelazione dell'umidità relativa dell'aria interna dopo la tripla differenziazione a lag 1 e grafico di cross correlazione dell' umidità relativa dell'aria esterna ed interna del vento dopo le opportune differenziazioni.

thermal and this may be due to the radiative proprieties of the wall itself, but the two variables are well cross-correlated and exhibit the same trend.

For RH the wall shows both a buffering effect and a decoupling effect: the internal environment does not suffer from the great variations in humidity of the external one.

3.2 Spring Period

A noticeable similarity in the relative trending of internal and external variables (Fig. 7) is found, showing that the wall has a constant effect that is not season-dependent. However this initial deduction is further analyzed as done for the fall period.

Even in spring the variables behave in the same way:

	Differenced External Air RH	Differenced Internal Air RH
Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity		
Statistic	0.024	0.051
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity		
Statistic	0.015	0.035
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Runs Test		
Statistic	2.145	2.3274
p-value	0.032	0.019
Result	H0 rejected	H0 rejected

Tab. 5 - Statistics of differenced air RH (Fall).

Tab. 5 - Statistiche per l'umidità relativa dell'aria differenziata (Autunno).

	Differenced External Air Temperature	Differenced Internal Air Temperature
Runs Test 'UD'		
Statistic	1.540	-1.418
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected

Tab. 6 - "Up-down" test statistics for differenced air RH (Fall).
Tab. 6 - Statistiche del test "up-down" per l'umidità relative dell'aria differenziata (Autunno).

there is a strong autocorrelation (Fig. 8) extending significantly up to even 12 time lags (for external wind speed) and it is still possible to detect the different behavior of the autocorrelation function between external and internal RH hinting again to the decoupling of the variables.

Complementary tests indicate a lack of stationarity of all the variables (only notable exception is the external air relative humidity which is stationary around a level, but not around a trend) (Tab. 7).

The most effective transformation for wind speed in term of variable stabilization and reduced loss of information, again, is applying the Box-Jenkins (Box and Jenkins, 1994) differentiation. The transformation proves to be effective and confirms the results found in fall (Fig. 9): the wall buffers the wind strength without decoupling the variables (cross correlation > 0.5 at lag 0).

After the differentiation the external wind speed results fully stabilized and deprived of significant memory (Tab. 8). The internal wind speed, instead, does not perform well with the standard runs test, but, since no evident trending or significant outliers could be detected, and since the 'UD' version of the test performs better, it has been chosen not to transform the variable again (Tab. 8).

The treatment of spring air temperature is particularly problematic: the application of differentiation once to internal air temperature and twice to air external temperature performs well in terms of elimination of autocorrelation and KPSS test. But this diverse differentiation of the two variables creates a problem in the cross-correlation analysis: the shifting due to differencing and the analysis of the cross-correlation function at different time lags produces an alternation in which peaks of high values shift towards peaks of low values (strong negative autocorrelation). Therefore both variables differenced twice are used (Fig. 10).

The KPSS Test shows that differencing is an effective transformation for both the internal and external air temperature (Tab. 9), while the internal air temperature does not perform well on the runs-test (not even in the 'UD' format). Still, since no outlier removal (both at 2 and 3 sigma), no trend fitting and no

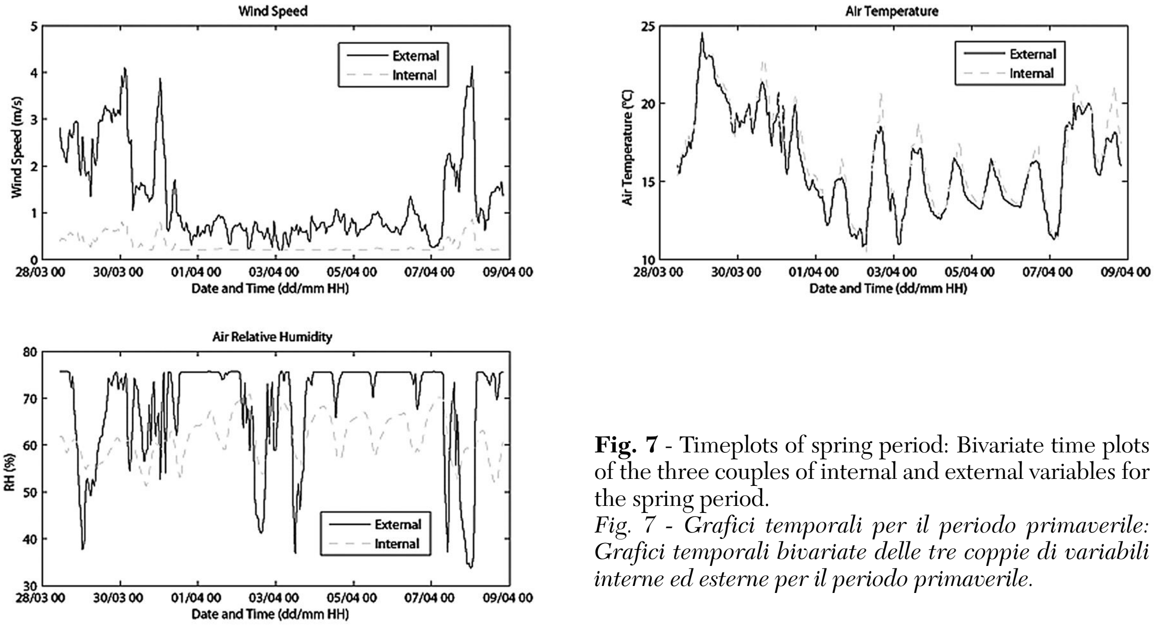

Fig. 7 - Timeplots of spring period: Bivariate time plots of the three couples of internal and external variables for the spring period.
Fig. 7 - Grafici temporali per il periodo primaverile: Grafici temporali bivariate delle tre coppie di variabili interne ed esterne per il periodo primaverile.

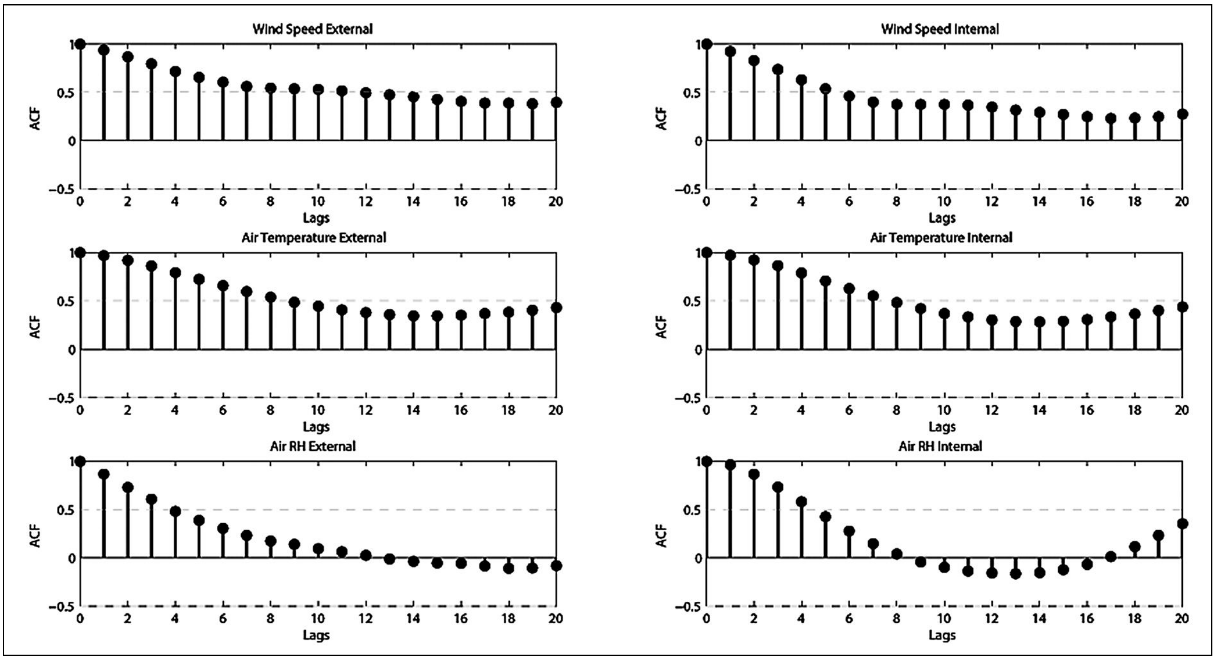

Fig. 8 - Spring autocorrelations: Autocorrelation functions for the three couples of internal and external variables in the spring period. The dashed grey line highlights the 0.5 autocorrelation level.
Fig. 8 - Autocorrelazioni primaverili: Funzioni di autocorrelazione per le tre coppie di variabili interne ed esterne per il periodo primaverile. La linea grigia tratteggiata evidenzia il livello 0.5 di autocorrelazione.

other transformation that maintained the physical significance of the variable changed the runs-test results, the differencing was considered the best transformation possible.

Differencing twice effectively reduces autocorrelations (Fig. 11) and gives a cross-correlation function that agrees well with the fall results.

RH performs well on all statistical tests after applying differencing three times. Again, differencing is proven the most effective technique: de-trending via polynomial fitting produces badly conditioned polynomials and no particular trend is detectable.

The external RH, though, required for some outliers to be removed: simply differencing three times produced an autocorrelation function which was still sensible at lag 1. The differenced variable is explored via lagged scatterplots which also indicate a strongly negative autocorrelation at lag 1 (Fig. 12).

	External Wind Speed	**Internal Wind Speed**	**External Air Temperature**	**Internal Air Temperature**	**External Air Relative Humidity**	**Internal Air Relative Humidity**
Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity						
Statistic	1.771	1.508	1.760	1.590	0.217	0.724
p-value	<0.01	<0.01	<0.01	<0.01	>0.1	0.011
Result	H0 rejected	H0 rejected	H0 rejected	H0 rejected	H0 not rejected	H0 rejected
Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity						
Statistic	0.987	0.730	0.832	0.767	0.163	0.423
p-value	<0.01	<0.01	<0.01	<0.01	0.03	<0.01
Result	H0 rejected	H0 rejected	H0 rejected	H0 rejected	H0 rejected	H0 rejected
Runs Test						
Statistic	-14.927	-14.081	-14.330	-14.670	-12.052	-14.208
p-value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Result	H0 rejected	H0 rejected	H0 rejected	H0 rejected	H0 rejected	H0 rejected

Tab. 7 - Statistics of Spring period.
Tab. 7 - Statistiche per il periodo primaverile.

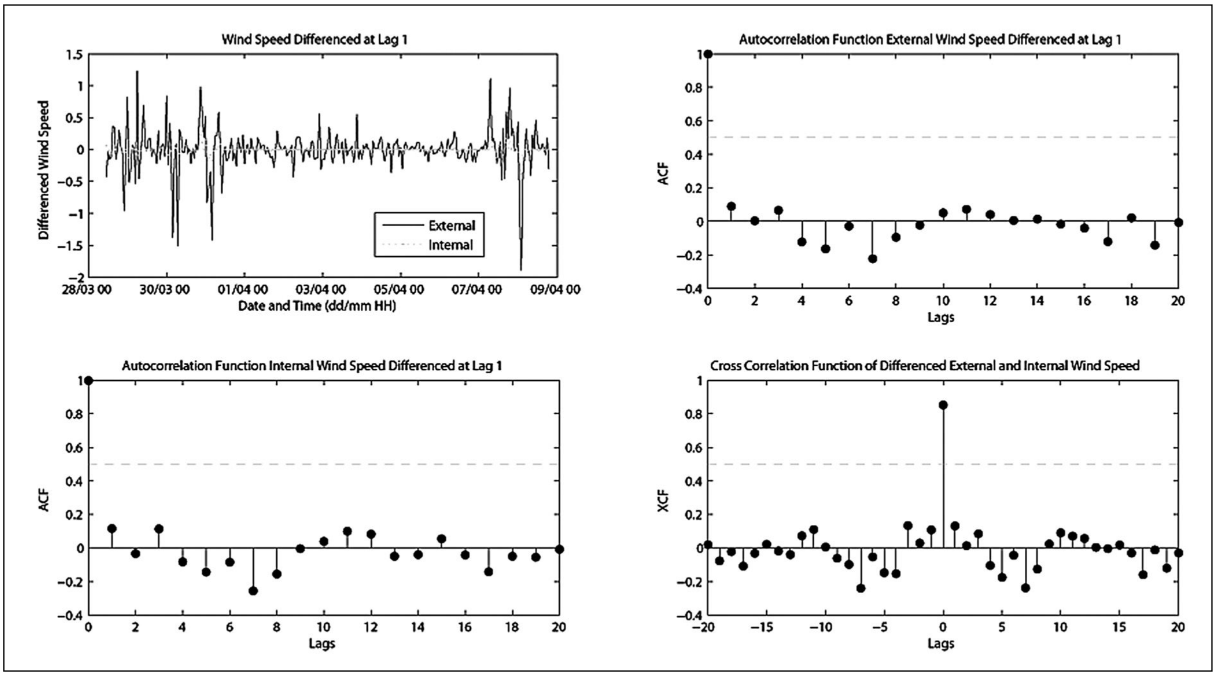

Fig. 9 - Plots of differenced wind speed (spring): From left to right and top to bottom: bivariate time plot of internal and external wind speed after differencing at lag 1, autocorrelation plot of external wind after differencing at lag 1, autocorrelation plot of internal wind speed after differencing at lag 1 and cross correlation plot of external and internal wind speed after differencing at lag 1.
Fig. 9 - Grafici per la velocità del vento differenziata (primavera): Da sinistra a destra e dall'alto in basso: grafico temporale bivariato della velocità del vento interna ed esterna dopo la differenziazione a lag 1, grafico di autocorrelazione della velocità esterna del vento dopo la differenziazione a lag 1, grafico di autocorrelazione della velocità del vento interna dopo la differenziazione a lag 1 e grafico di cross correlazione della velocità esterna ed interna del vento dopo la differenziazione a lag 1.

The exploration of the lagged scatterplots at lag 1 shows that there's a strongly negative autocorrelation. Afterwards a conservative outlier removal is applied: values exceeding 3 times the standard deviation are found and substituted with the mean of the two adjacent values. This procedure removes the autocorrelation at lag 1 (Fig. 13).

The autocorrelation function changes accordingly, showing a removal of significant autocorrelation at lag 1 (Fig. 14).

	Differenced External Wind Speed	**Differenced Internal Wind Speed**
Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity		
Statistic	0.043	0.018
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity		
Statistic	0.015	0.017
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Runs Test		
Statistic	-0.905	-4.394
p-value	>0.1	<0.01
Result	H0 not rejected	H0 rejected
Runs Test 'UD'		
Statistic	-1.226	-1.887
p-value	0.220	0.06
Result	H0 not rejected	H0 not rejected

Tab. 8 - Statistics of differenced wind speed (Spring).
Tab. 8 - Statistiche per la velocità del vento differenziata (Primavera).

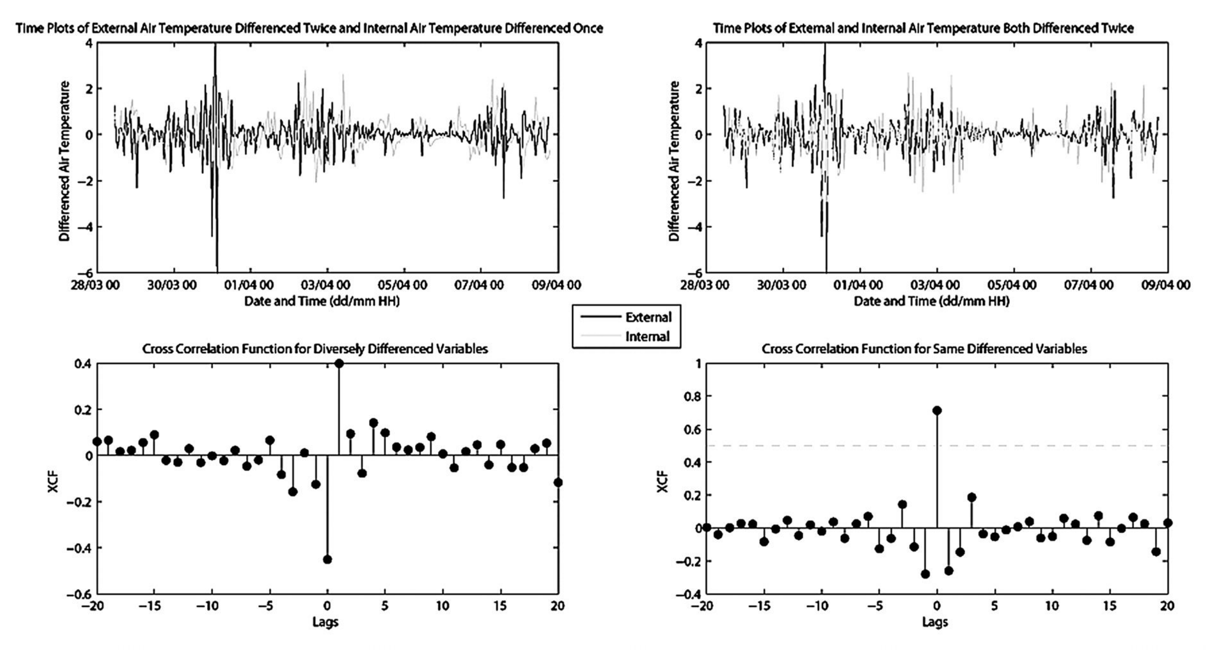

Fig. 10 - Diverse differencing of air temperature: Effect of diverse differencing over the internal and external air temperature.
Fig. 10 - Varie differenziazioni della temperatura dell'aria: Effetto della diversa differenziazione sulla temperatura dell'aria interna ed esterna.

After the aforementioned procedures the "stabilized" couple of external and internal RH can be compared producing results that agree well with those found in the fall period. The internal RH values are buffered by the wall and there is a decoupling of the RH between the external and the internal environment (no significant cross correlation at all time lags) (Fig. 15).

After the aforementioned transformation, the variables performed very well in all the statistical tests confirming lack of autocorrelation and trending (Tab. 10).

The results of the spring analysis are consistent with those found in the fall: even in spring the wall acted in the same manner so it is reasonable to say that its effects are not season-dependent.

Tab. 9 - Statistics of differenced air temperature (Spring).
Tab. 9 - Statistiche per la temperatura dell'aria differenziata (Primavera).

	Differenced External Air Temperature	**Differenced Internal Air Temperature**
Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity		
Statistic	0.009	0.012
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity		
Statistic	0.008	0.012
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Runs Test		
Statistic	2.860	4.168
p-value	<0.01	<0.01
Result	H0 rejected	H0 rejected
Runs Test 'UD'		
Statistic	1.903	2.337
p-value	0.057	0.02
Result	H0 not rejected	H0 not rejected

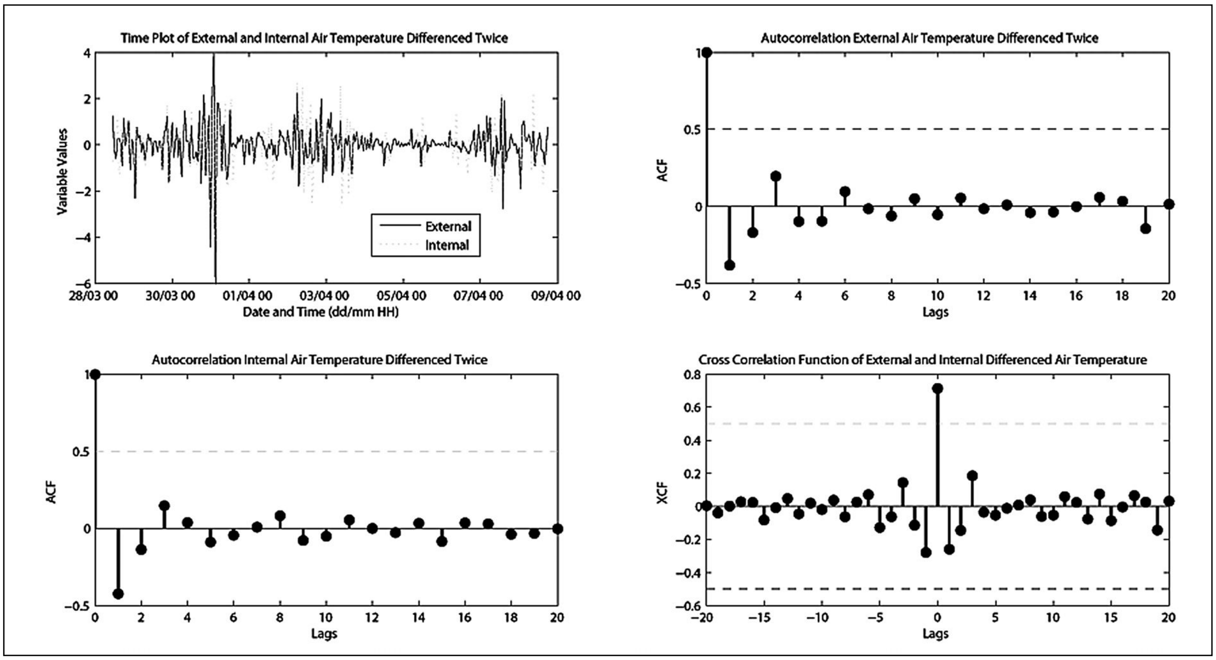

Fig. 11 - Plots for differenced air temperature (spring): From left to right and top to bottom: bivariate time plot of internal and external air temperature differenced twice, autocorrelation plot of air temperature differenced twice, autocorrelation plot of internal air temperature after differencing twice and cross correlation plot of external and internal air temperature after differencing twice.

Fig. 11 - Grafici per la temperatura dell'aria differenziata (primavera): Da sinistra a destra e dall'alto in basso: grafico temporale bivariato della velocità del vento interna ed esterna dopo la differenziazione a lag 1, grafico di autocorrelazione della velocità esterna del vento dopo la differenziazione a lag 1, grafico di autocorrelazione della velocità del vento interna dopo la differenziazione a lag 1 e grafico di cross correlazione della velocità esterna ed interna del vento dopo la differenziazione a lag 1.

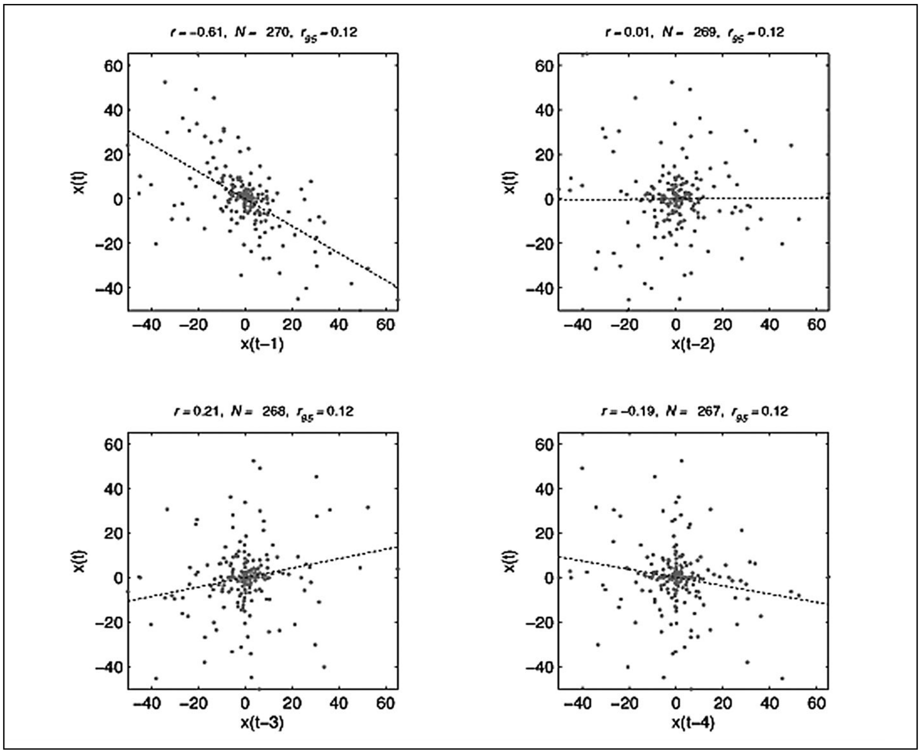

Fig. 12 - Lagged scatterplots of differenced RH (spring): lagged scatterplots of the external air relative humidity differenced three times. From top to bottom and left to right: scatterplot at lag 1, 2, 3 and 4.

Fig. 12 - Scatterplots per l'umidità relativa dell'aria differenziata (primavera): scatterplots con lag temporali dell'umidità relativa dell'aria esterna differenziata tre volte. Dall'alto verso il basso e da sinistra a destra: scatterplot al lag 1, 2, 3, e 4.

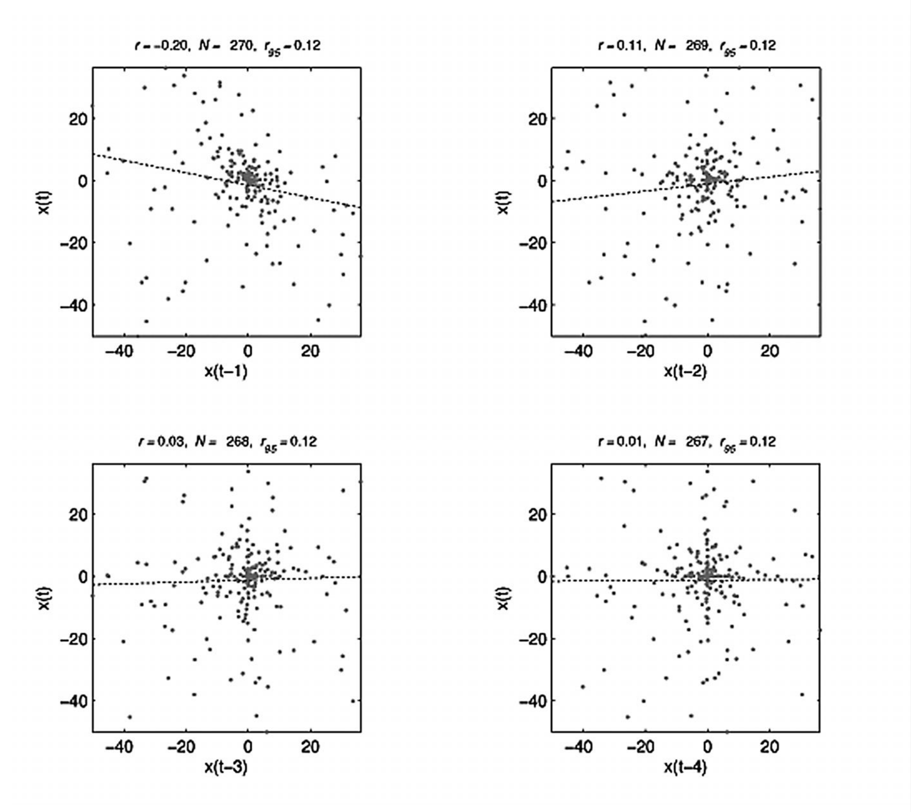

Fig. 13 - Effect of outlier removal on scatterplots: lagged scatterplots of the external air relative humidity differenced three times and with outliers removed. From top to bottom and left to right: scatterplot at lag 1, 2, 3 and 4.
Fig. 13 - Effetto della rimozione degli outliers sugli scatterplots: scatterplots con lag temporali dell'umidità relative dell'aria esterna differenziata tre volte e con gli outliers rimossi. Dall'alto al basso e da sinistra a destra: scatterplot al lag 1, 2, 3 e 4.

3.3 Spectral Analysis

The effect of differencing is effective in redistributing the variance over various frequencies, with the exception of air temperature which still maintains a spectrum showing traces of red noise with main frequency peak around 0.043. This indicates a period of about 24 hours, indicating a daily cycle of temperature, as it is perfectly expectable. Raw periodograms for the unmodified (left column) and stationary (right column) variables in the fall period are resumed in Fig. 16. Even in spring (Fig. 17) differencing redistributes variance over various frequencies ("red noise" removal) and it shows that the more time the differencing is applied the stronger the effect. Spring air temperature (even in the non-differenced form) loses its strong 0.043 spike (even if it's still visible). The difference in the external and internal air RH spectra is again a proof of a different humidity regime between the external and internal environment.

	Differenced External RH	**Differenced Internal RH**
Kwiatkowski-Phillips-Schmidt-Shin Test for Level Stationarity		
Statistic	0.064	0.012
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Kwiatkowski-Phillips-Schmidt-Shin Test for Trend Stationarity		
Statistic	0.049	0.008
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected
Runs Test		
Statistic	-0.332	0.489
p-value	>0.1	>0.1
Result	H0 not rejected	H0 not rejected

Tab. 10 - Statistics of differenced air RH (Spring).
Tab. 10 - Statistiche per l'umidità relativa dell'aria differenziata (Primavera).

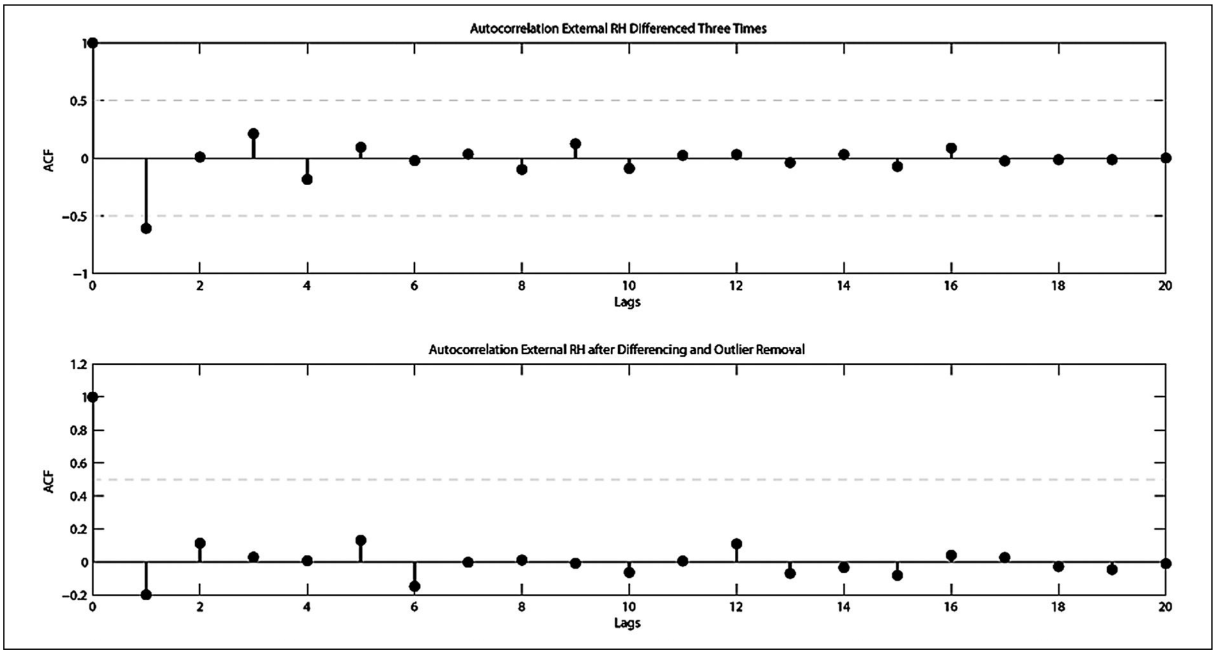

Fig. 14 - Effect of outlier removal on autocorrelation function: Autocorrelation function of external RH after differencing (top) and external RH after differencing and outlier removal (bottom).
Fig. 14 - Effetto della rimozione degli outliers sulla funzione di autocorrelazione: Funzione di autocorrelazione dell'RH esterna dopo la differenziazione (in alto) e dell'RH esterna dopo la differenziazione e la rimozione degli outliers (in basso).

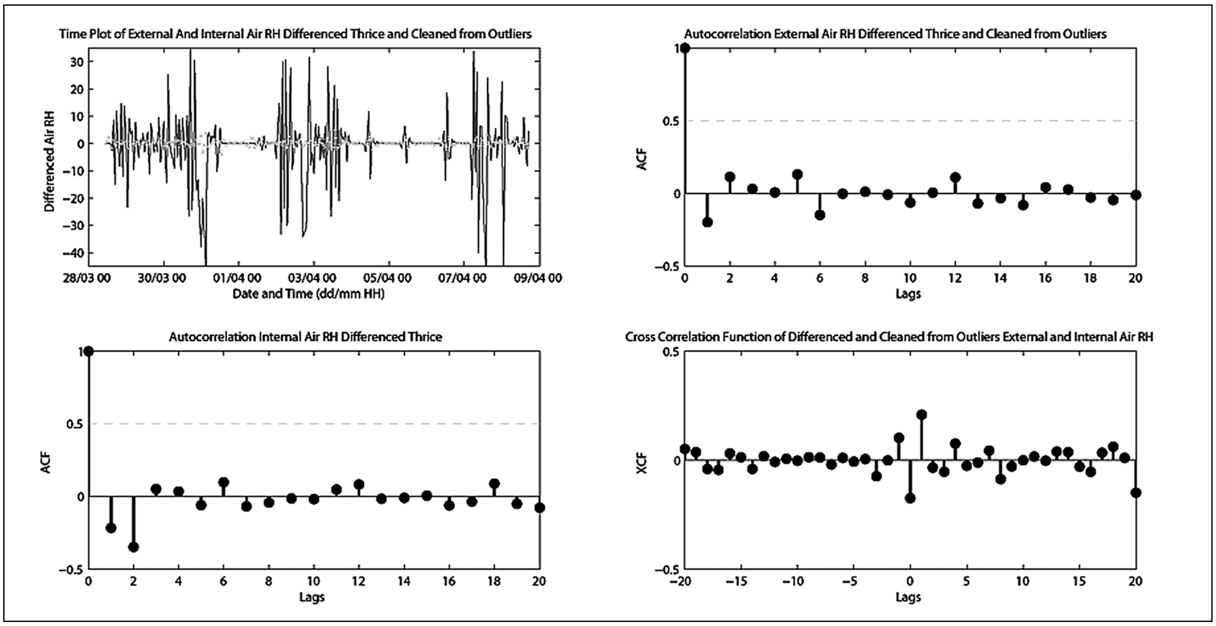

Fig. 15 - Plots for differenced air RH (spring): From left to right and top to bottom: bivariate time plot of internal and external air RH differenced thrice and cleaned from outliers, autocorrelation plot of external air RH differenced thrice and cleaned from outliers, autocorrelation plot of internal air RH after differencing thrice and cross correlation plot of external and internal air RH after differencing three times.
Fig. 15 - Grafici per l'umidità relativa dell'aria differenziata (primavera): Da sinistra a destra e dall'alto in basso: grafico temporale bivariato dell'umidità relativa dell'aria interna ed esterna differenziata tre volte e ripulita dagli outliers, grafico di autocorrelazione dell'umidità relative dell'aria differenziata tre volte e ripulita dagli outliers, grafico di autocorrelazione dell'umidità relativa dell'aria interna differenziata tre volte e grafico di cross-correlazione dell'umidità relativa dell'aria esterna ed interna differenziata tre volte.

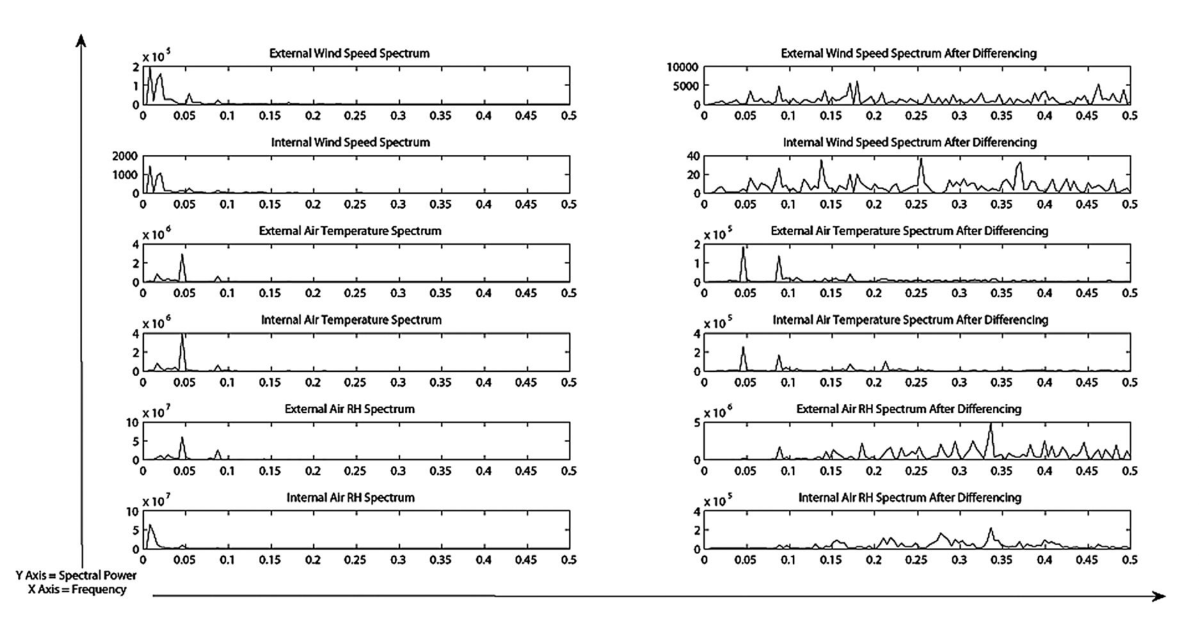

Fig. 16 - Spectra for fall season: Spectra for fall season.
Fig. 16 - Spettri per la stagione autunnale: Spettri relativi alla stagione autunnale.

4. CONCLUSIONS

The dry-stone landscape of Pantelleria includes a number of unique cultural adaptations that have been developed under the pressure of the environmental limitations. To date, about 80% of the island of Pantelleria is terraced and more than 500 Pantesco gardens are present, even though it is currently endangered by the gradual abandonment of the

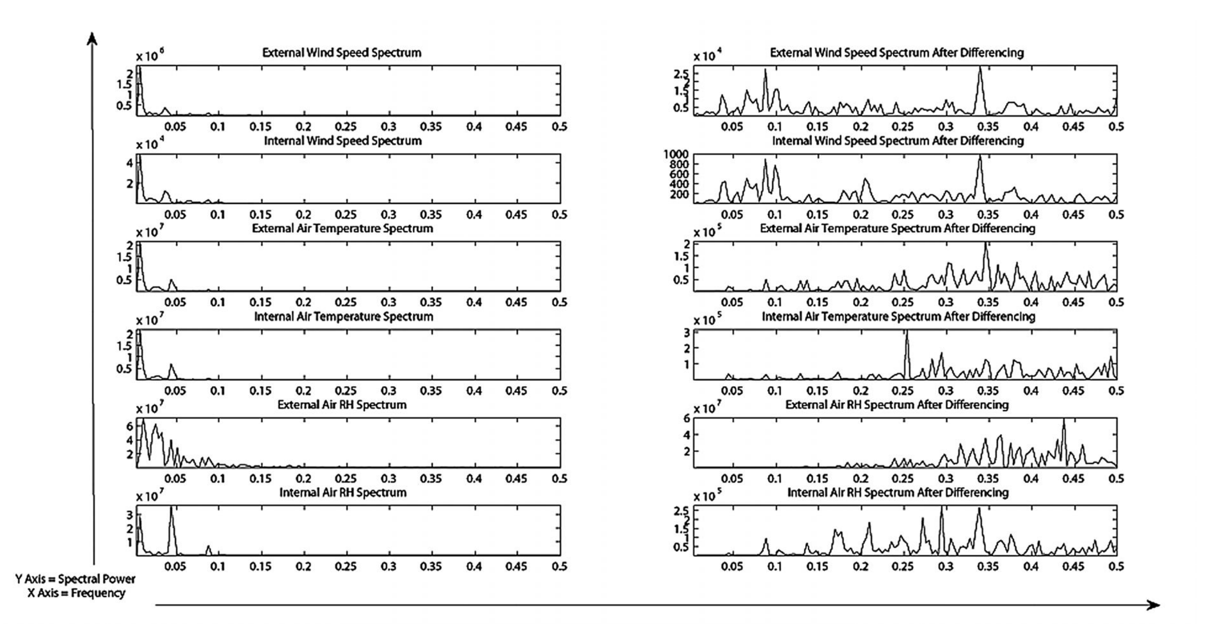

Fig. 17 - Spectra for spring season: Spectra for spring season.
Fig. 17 - Spettri per la stagione primaverile: Spettri relativi alla stagione primaverile.

terraces and buildings located in the most difficult conditions.

The strong winds and water scarcity have been the most determinant in shaping cultural systems. Sheltering from winds by confining plant canopies within the boundary layer of the dry-stone walls of gardens and terraces and inducing, as in grapevine, olive and capers, tree dwarfing by pruning and/or plant training systems can be identified as a common general adaptive farming criterion.

The Pantesco garden is a unique and noticeable example of combination of work of human and nature, in which natural obstacles have been overcome in a sustainable way to create an opportunity for growing high nutritional value citrus fruits. A chance to locally supply vitamins to people living in an island matches with the symbolic value, for people who moved from the bigger Sicily where lemon and orange are the main traditional species, to bring to their new living place these same crops and be able to farm in a more hostile environment.

The analysis carried out in this paper has shown that the stone wall enclosing the Giardino Pantesco has indeed an effect, different from variable to variable, that mitigates weather variables, otherwise compromising, and allows the growth of the encircled citrus tree. This ancient traditional agronomic technique can give interesting insights and possible applications even nowadays. The kind of structure, its dimension, and the use of local material to mitigate the effect of climate are potentially efficient tools for maintaining local biodiversity and allow cultivation of species otherwise unsuitable for the island climate. In addition, local biodiversity is implemented by the cracks and fissures between the stones that host a wealth of organisms, offering both a safe shelter and a favorable microclimate.

The statistical framework presented in the context of this paper results effective in reducing autocorrelation and "stabilizing" the examined time series, allowing for a comparison not biased by variable memory. This methodology is based on short data sets and simple measurements of meteorological variables and can be hence proposed as a tool for studying and comparing local situations in which microclimate modifications have been created or naturally occur. This can be part of a greater design for better understanding the physical basis of the traditional knowledge in protected farming, and for a sounder safeguard policy of preservation and restoration of traditional rural and agricultural landscape.

5. ACKNOWLEDGEMENTS

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Meeting farmers' needs for agrometeorological services: A review with case studies

Part I: Introduction and history

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Abstract: *Meeting the needs for agrometeorological services, including agroclimatological services, in the livelihood of farmers is the focus of this paper in four Parts. This ideally leads to agrometeorological services for response farming with irrigation scheduling, early warnings, microclimate manipulation, and application of weather and climate forecasts in a changing and increasingly variable climate. In this first Part, some historical aspects are dealt with in the introduction, particularly regarding the definition and scope of agrometeorology. What has recently been said about such services is exemplified, including examples from South Africa, Cuba, Zambia and India.*
Keywords: *agrometeorogical service, Cuba, India, South Africa, Zambia.*

Riassunto: *Soddisfare le esigenze per i servizi agrometeorologici, compresi i servizi agroclimatici, nel sostentamento agli agricoltori è il centro di questo lavoro in quattro parti. Questo porta i servizi agrometeorologici ad essere la risposta ideale per l'agricoltura con la pianificazione dell'irrigazione, con le prime avvertenze, con la manipolazione del microclima e l'applicazione delle previsioni meteorologiche e climatiche in un clima sempre più mutevole e variabile. In questa prima parte, nella premessa sono trattati alcuni aspetti storici, soprattutto per quanto riguarda la definizione e lo scopo dell'agrometeorologia. Ciò che è stato detto di recente su tali servizi è esemplificato, con esempi dal Sud Africa, Cuba, Zambia e India.*
Parole chiave: *servizi agrometeorologici, Cuba, India, Sud Africa, Zambia.*

1. INTRODUCTION

1.1 History

In the past 40 years, the fields where agricultural meteorology is applied grew extensively (WMO, 2006). With an increasing rate of application in the developing world, the definition of agricultural meteorology had to be widened to accommodate the conditions in developing countries, with their more abundant weather and climate disasters and their endangered environments. This was the first gradual widening of priorities in agricultural meteorology (Stigter, 2008a; 2009a).

In his Roving Seminars in southern countries and elsewhere (Fig. 1), Stigter teaches that society and economics do not equate to agricultural meteorology, but that the consequences and use (that is, management) of water, radiation/heat and air in society and economics, as far as the agricultural production environment is concerned, slowly became an undercurrent in agricultural meteorology (WMO, 2006; KNMI, 2009; Stigter, 2010a). Translated to poorer countries, the socio-economic aspects of elements such as multiple cropping, irrigation, storage, agroforestry, floods, drought, erosion and desertification, frost and wind protection, simple artificial growth conditions, sustainable farming and related farmers' income generation became additional priorities in that widening undercurrent of agricultural meteorology. This was a second gradual widening of the subjects associated with agricultural meteorology (Stigter, 2008a; 2009a).

The widening of the definition of agricultural meteorology and the second widening of the scope of related subjects have largely been missed by training and education, and more so in developing countries (Stigter, 2009a). This can be explained through a simple conceptual and diagnostic framework that was some years ago published in a guest editorial (Stigter, 2007a) and is also used in Stigter (2009a; 2010a) and Stigter et al. (2010).

By such a new definition (as worded by Stigter and Walker in a 2008 folder of the University of the

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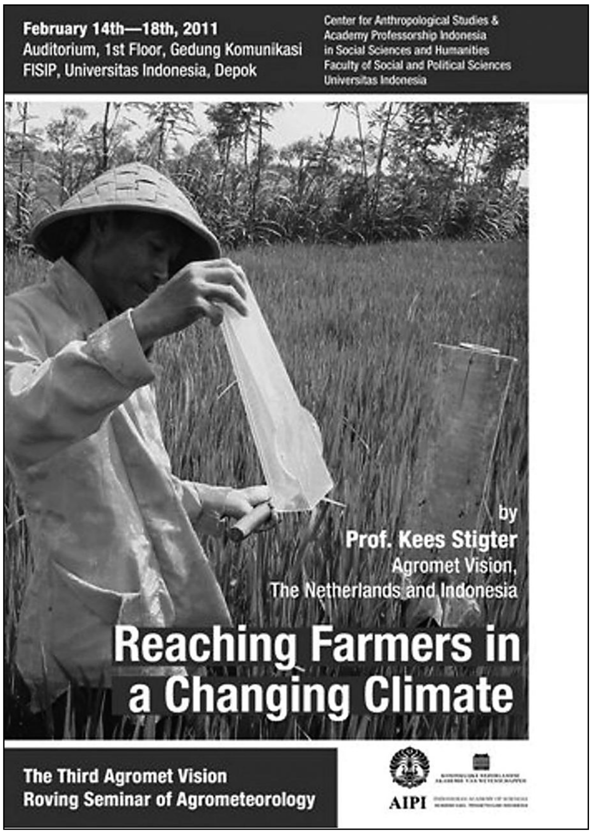

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Universitas Indonesia

by
Prof. Kees Stigter
Agromet Vision,
The Netherlands and Indonesia

Reaching Farmers in a Changing Climate

The Third Agromet Vision
Roving Seminar of Agrometeorology

AIPI

Fig. 1 - An announcement of one of Stigter's Roving Seminars in Indonesia. [Photo Yunita Winarto. Design Maximilian Aji Wijoyoseno]
Fig. 1 - Annuncio di uno dei Seminari itineranti di Stigter in Indonesia. [Foto Yunita Winarto. Progettazione Maximilian Aji Wijoyoseno]

Free State (UFS), Bloemfontein, South Africa), agrometeorology and agroclimatology investigate adaptation strategies to weather and climate in raising crops, trees, livestock and fish. They study water, heat, air and related biomass development in the agricultural production environment, including disasters, and their socio-economic consequences for farmers as decision makers. This ideally leads to agrometeorological services for response farming with irrigation scheduling, early warnings, microclimate manipulation, and application of weather and climate forecasts in a changing and increasingly variable climate (Stigter and Walker in the same 2008 UFS folder).

1.2 Agrometeorological services

Meeting the needs for agrometeorological services, including agroclimatological services, in the livelihood of farmers is the focus of this paper. Such services were defined, exemplified and explained in Stigter (2007a) (List I) and Stigter (2010a, 2011a). In summary, they cover the development of meteorological and climatological information suitable to give support, as services, to the whole set of farmer tactical and strategic decisions. They go from guiding quantitative rainfall measurements by farmers in their plots to new knowledge in learning services related to seasonal climate scenarios and understanding yield differences between fields, seasons and years (e.g. Winarto and Stigter, 2011; Stigter *et al.*, 2013).

List I. Agrometeorological services as defined in Stigter (2007a)

- The products of agroclimatological characterization, obtained with whatever methodologies;
- Advises such as in design rules on above and below ground microclimate management or manipulation, with respect to any appreciable microclimatic improvement: shading, wind protection, mulching, other surface modification, drying, storage, frost protection, etc.;
- Advisories based on the outcome of response farming exercises, from sowing window to harvesting time, using climatic variability data & statistics of a recent past or simple on-line agrometeorological information;
- Establishing measures reducing the impacts and mitigating the consequences of weather and climate related natural disasters for agricultural production;
- Monitoring and early warning exercises directly connected to such already established measures in agricultural production, to reduce the impacts and to mitigate the consequences of weather and climate related natural disasters for agricultural production;
- Climate predictions and forecasts and meteorological forecasts for agriculture and related activities, on a variety of time scales, from years to seasons and weeks, and from a variety of sources;
- Development and validation of adaptation strategies to increasing climate variability and climate change and other changing conditions in the physical, social and economic environments of the livelihood of farmers;
- Specific weather forecasts for agriculture, including warnings for suitable conditions for pests and diseases and/or advises on countervailing measures;
- Advices on measures reducing the contributions of agricultural production to global warming and keeping an optimum level of non-degraded land dedicated to agricultural production;

- Proposing means of direct agrometeorological assistance to management of natural resources for development of sustainable farming systems in technological advances with strong agrometeorological components.

Stigter therefore also teaches in his Roving Seminars that next to "science and technology" and "the understanding of local adaptive strategies and innovations", the four cornerstones of what is needed to fight poverty, empower people and enhance people's dignity through life long education, are completed by agrometeorological services as "policies" and "the high internal input": the farmers themselves (KNMI, 2009). Agrometeorological services in developing countries have to shoulder greater responsibilities due to larger population pressure and changing modes of agricultural practices (Fig. 2).

1.3 Local problems and conditions

More and more demands pertaining to agrometeorological information and services will be coming from the farming communities in the future, on technologies, farming systems patterns and practices (better including multiple cropping, see Stigter, 2010a), water management, weather based pest and disease control etc., preferably with local innovations as starting points. Thus the future challenges include the necessity to emphasize a bottom up approach so that forecasts, specific advisories and contingency planning serve even the small farmers for applications in their planning and day-to-day agricultural operations (Stigter *et al.*, 2010, Stigter, 2011a). See for understanding the actual problems in developing countries the Cuban example of Box 1. For an example of a solution developed in Zambia, see Box II. A local situation there is pictured in Fig. 3.

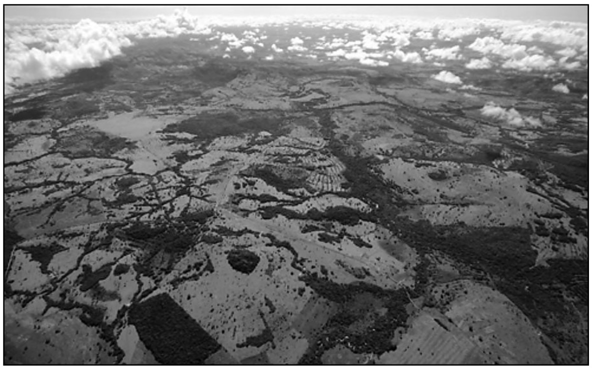

Fig. 2 - The goal of this work is to promote policies that meet the needs of rural poor communities worldwide. [*Courtesy the Ford Foundation.* http://www.fordfoundation.org/issues/ sustainable-development/climate-change-responses-that-strengthen-rural-communities]

Fig. 2 - L'obiettivo di questo lavoro è di promuovere politiche in grado di soddisfare le esigenze delle comunità rurali povere in tutto il mondo. [Per gentile concessione della Fondazione Ford http://www.fordfoundation.org/issues/sustainable-development/climate-change-responses-that-strengthen-rural-communities]

Fig. 3 - Complex growth and livelihood conditions in Zambia that must be met by agrometeorological community services (from Nanja, 2011).

Fig. 3 - Complesse condizioni di crescita e sostentamento che devono essere affrontati dai servizi della comunità agrometeorologica in Zambia (Nanja, 2011).

2. LIVELIHOOD OF FARMERS

Agrometeorological services in developed countries focus on the provision of environmental data and information to national policy and decision makers. They do that in support of sustained food production, sustainable development, carbon sequestration in agro-ecosystems and land management practices that affect exchange processes of greenhouse gasses. Because developed countries may have or develop technology to initially adapt more readily to climate change and climate variability, technology transfer may play a certain role. But local innovations, such as those in multiple cropping, remain most important for application under the very different conditions in developing countries (Stigter *et al.*, 2010, 2013; Stigter and Winarto, 2012).

A good definition of livelihood, from Chambers (1990), is: "means to gain adequate stocks and flows of food and cash to meet basic needs, together with reserves and assets to offset risks, ease shocks, and meet contingencies". The same source argued that in practice the livelihood strategies of poor people, including resource-poor farmers, are often complex and diverse and can be different in the same village (Chambers, 1990). Stigter *et al.* (2007) showed that four different income-levels of farmers in China treated the technological and related information differently, and their levels of satisfaction were different too. They also appeared to receive the information largely through different channels.

Because operational agrometeorology has to be carried out in the livelihood of farmers, we must be on speaking terms with extensionists, anthropologists, and other agricultural and social scientists as well as development economists (Stigter, 2010a). The bridge between our fields of work and theirs was very well set up by Robert Chambers in his "Microenvironments Unobserved" (Chambers, 1990). His approach explains why scientists, if at all interested in applications of their findings, often come up with wrong solutions presented along the wrong communication channels, and for the exceptional potentially suitable answers in ways that are insufficiently client friendly. He explained then, and Stigter (2010a) repeated this with new arguments, that the consequences of poverty and vulnerability are not clearly understood by scientists, nor are the possibilities within farmers'

Box I - Cuban experience in the communication of local agrometeorological information and services

Transfer of agrometeorological information to farmers is done in different ways. Meteorological Services use different options, such as periodical bulletins (printed or web), mass media: TV, radio or newspapers and/or e–mails. Perception studies developed in areas around an agrometeorological station in Villa Clara[1], central region of Cuba, showed that meteorological information was useful to most of the farmers. However, a considerable portion of the farmers was unaware of the concepts and scope of agricultural meteorology.

Moreover, it was found that the national television news (NTV) and/or national radio stations were selected more often as the first source of weather information, followed by the local television channel (TELECUBANACÁN) and/or local radio. Respondents to this questionnaire, all of them farmers, this way always kept themselves informed on the weather situation and used that information mostly for planning purposes. What can we do to improve on that situation? How to convey more specific agrometeorological information safely and understandably to producers? To solve this problem, our conditions demand to make a differentiation, because obviously messages aimed at managers of agricultural enterprises differ from those whose receptors are individual producers. In Cuba these actors generally do not have access to electronic networks and in many cases even lack telephones, to mention two elements considered limitations for the design or establishment of any information system.

First, in the case of agricultural managers, the agrometeorological services should be simple for their proper assimilation and they must be used frequently to facilitate decision-making and planning. The experience with communication of local agrometeorological information showed that e-mail can be a good choice, but this has some essential limitations such as: I) customer should be a user of an e-mail provider: This prerequisite is not always met; II) excessive personalization of information: the information reaches only the recipient, without opportunity for all others interested to get documented, III) additional costs for information transport, depending on the connection fee of the company to his mail provider, IV) limited operation: arrival of the information at its destination depends on the connection between transmission and reception and V) improper delegation of responsibility: part of the responsibility for the speed and quality of information would depend on third parties.

These considerations led to the creation of a system for information transport, the so called *Remote Web System*, which was established on the basis of a link *point to point* (P2P), which has certain technical advantages, including: i) updating is independent of user intervention, ii) provides a framework for the review of information, iii) allows for secure information exchange between supplier and customer and iv) the supplier has all the statistical use and it is easy to determine any violation of the regulations. *Remote Web System* contributed to the solution, but only partially because lack of telephone lines and modems capable to cover all customers in a minimum of time, was, next to website design (requires a more "flexible" website) and training, the most important constraint. The problem for individual farmers remains because we aimed at farm managers. Certainly, we are not satisfied! What to do?

It imposes the need to "downscale" the role of agrometeorological stations, which should not only be "centers for collecting data and information" but also something like a referral and consultation center at the local level. If we take a look at our surface weather stations, it should be closest to the agricultural producers. It somehow should reach those places we can not "see" and have the badly wanted possibility to be in direct contact with farmers. Daily we witness that data and agrometeorological information "are lost" without use of their potentials. The idea then arose to use the local radio transmitters as spokesmen of our message. Of course, to reach this goal, the first step was negotiating with the broadcasting authorities to obtain time blocks - in our case free of charge – at times that farmers can listen. At the same time we designed and distributed a new monthly agrometeorological newsletter. Radio ensures greater "visibility" of the agrometeorological information, but how do farmers absorb this information? Do they know how to interpret it? Does it meet their needs? To answer these questions requires evaluation of receipt and use of these services, increasingly focused on the local farmers. In this process, agrometeorologists should be assisted by social communication specialists to ensure the success of their endeavours with the right differentiation (see also Stigter et al., 2007).

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[1] Further information on this study can be found at *http://ram.meteored.com/numero46/informacion-agrometeorologica.asp* (only in Spanish).

Box II - Use of local community radio to distribute seasonal farmer advice in Zambia

The farmers in the Mujika area requested further information about the climate and how it affects their crops. Through participatory needs assessments at several villages in the area, it was established that there are a range of on-farm decisions that are dependent on the environment. For example, they have a range of lands available for cropping from clay soils in low lying areas to sandy soils in the higher areas. There are also a range of maize seeds available of different growing period length for short, medium and long season varieties. Each year the farmers must decide which seed to plant; as well as where and when to plant them. The existing local community radio station in Monze agreed that the agrometeorologist can broadcast farmer-climate (weekly weather and seasonal climate) information in a regular slot once a week. Some of the broadcasts took the form of a role play with several people who discuss the climate messages and their farm activities according to the appropriate time of the year. For instance, before the summer rains begin, the role players discuss the tasks needed to prepare for the maize planting and how the latest seasonal forecast influences their cropping decisions.

The policy support for these agrometeorological services came from the provincial government level from the Southern Province branch of the Department of Meteorology in Livingstone. They were able to supply some tape recorders to some of the farmer study group leaders who then recorded the radio programme each week. Later, these recorded radio programmes were then replayed during the farmer group meetings. The farmers then discussed the information and used the climate prediction to make a decision about which maize variety to plant and where to plant it. The Southern Province Department of Meteorology also supported the personnel to make the radio programmes and to visit some of the farmer meetings. The Meteorology Department made it their policy to support this type of information dissemination to grassroots level in the community (Nanja, 2010).

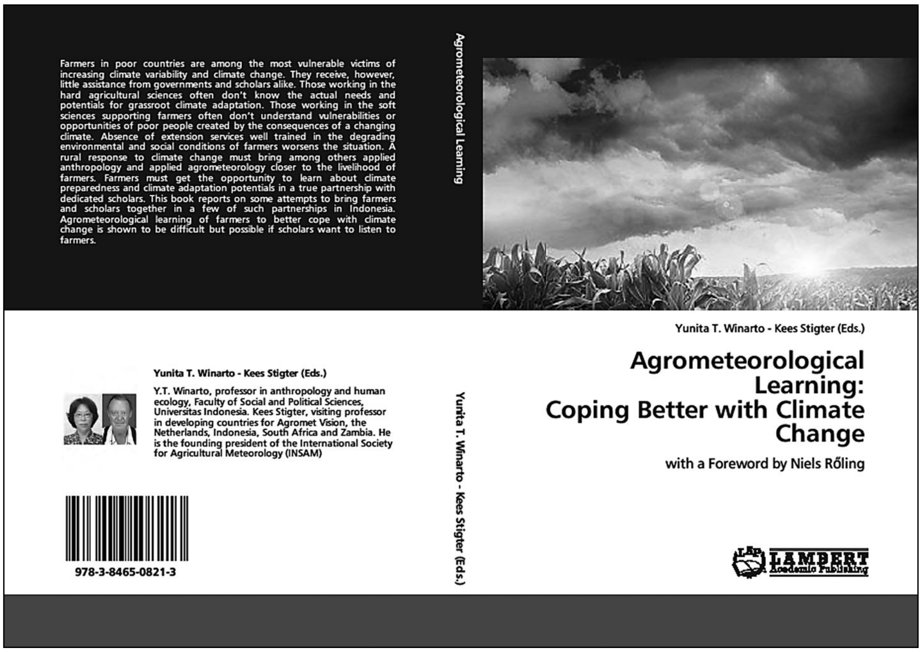
actual existence. Indian experience with the latter is found in Box III.

Much has already been reported by the senior author of this paper on the livelihood context of farmers' needs for agrometeorological services, also recently (e.g. Stigter, 2007a; 2007b; 2007c; 2008a; 2008b; 2008c; 2008d; 2009b; 2010a; 2010b, 2011a). We report these days also on farmer learning processes in agrometeorology (Fig. 4, Winarto and Stigter, 2011; Nanja, 2011). To further characterize this context, we therefore use in Part II of this paper in four parts, some recent reviews in which we recognize that of our own approaches. In Part III we define two connecting principles of these reviews for the future in (I) agroforestry as multifunctional agriculture (e.g. Stigter, 2011a; Stigter *et al.*, 2011) and (II) communication between stakeholders and extension (e.g. Stigter and Winarto, 2012; Stigter *et al.*, 2013). Subsequently we will argue in this Part III that in new educational commitments, when well institutionalized (Stigter, 2009b), the understanding of farmers' needs can be extended and handled to redress their livelihood situation using agrometeorological services (Stigter, 2011). Part IV of this

Box III. Livelihood experience with agrometeorological services from India

Recent studies in India showed that economic impact of an Agro-Advisory Service (AAS) based on weather forewarning is significant and benefited the AAS farmers to a large extent through weather-tuned farming. AAS farmers reaped more yield when compared to non-AAS farmers owing to technical guidance on all cultivation aspects, especially selection of varieties, timely application of fertilizer/pesticides, inputs saving in terms of water, manpower, electricity, and fuel through proper irrigation scheduling (Prasad Rao and Manikandan, 2008; Kushwaha *et al.*, 2008).

A farmer may want to use forecasts for decisions at a number of scales; in order to manage farm decisions; to plan water resource management depending on how much rain is expected in the catchment or to use the expected national food supply forecast to decide on the investment in inputs (Das *et al.*, 2010). This illustrates that although users may operate primarily at one scale, their decision-making may depend on information from a variety of scales and so varying levels of forecast skill might be acceptable. Despite the scale of action and decision-making, it is paramount to accompany improved dissemination with improved explanations of forecast characteristics and limitations. Although seasonal forecasts are expected to be used more frequently in the future, the cost of taking precautions (based on the forecast) must be weighed against the savings that the precautions would bring if the unwanted climate event occurred. Users of seasonal forecasts could, for example, be more actively engaged in economic evaluation assessments (Richardson, 2000) to get an idea of the potential rewards and penalties accrued in unfavourable weather situations.



Farmers in poor countries are among the most vulnerable victims of increasing climate variability and climate change. They receive, however, little assistance from governments and scholars alike. Those working in the hard agricultural sciences often don't know the actual needs and potentials for grassroot climate adaptation. Those working in the soft sciences supporting farmers often don't understand vulnerabilities or opportunities of poor people created by the consequences of a changing climate. Absence of extension services well trained in the degrading environmental and social conditions of farmers worsens the situation. A rural response to climate change must bring among others applied anthropology and applied agrometeorology closer to the livelihood of farmers. Farmers must get the opportunity to learn about climate preparedness and climate adaptation potentials in a true partnership with dedicated scholars. This book reports on some attempts to bring farmers and scholars together in a few of such partnerships in Indonesia. Agrometeorological learning of farmers to better cope with climate change is shown to be difficult but possible if scholars want to listen to farmers.

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Agrometeorological Learning

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Agrometeorological Learning: Coping Better with Climate Change

with a Foreword by Niels Rőling

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Fig. 4 - Learning of relevant agrometeorology by farmers is now getting special attention in new extension commitments (Winarto and Stigter, 2011).
Fig. 4 - L'apprendimento d'informazioni pertinenti l'agrometeorologia da parte degli agricoltori sta acquisendo particolare attenzione per una nuova estensione degli impegni (Winarto e Stigter, 2011).

paper illustrates our approach with a series of historical case studies of best examples of agrometeorological services.

3. ACKNOWLEDGEMENTS

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The Global Federation of Agrometeorological Societies is launching

Federica Rossi[1]

Presidents or delegates of most of the existing National Agrometeorological Societes convened in Jeju, Republic of Korea, on November 5, 2013 to discuss about the opportunity to create a Federation, tentatively named GlobalFAMS. Dr. Federico Spanna, President of the Italian Society of Agrometeorology participated to the meeting, where a very active and propositive discussion was held.

It was agreed that, since the complexity of drivers facing world's agriculture, there are significant challenges for developing proactive national and international actions that support the wider implementation of more sustainable, environmental-friendly and efficient land use and agricultural production across countries of the world with varying degrees of socio-economic development. All sectors of the agricultural production chain are players in the process to make such transition happen. This includes famers as primary producers of food to data providers, technical and scientific developers of resource tools as NMHSs, academic and research institutions, and local, national and global policy makers. International cooperation, based on resources, knowledge sharing, and mutual understanding is absolutely essential to bring proactive tangible global advancement towards uplifting the living standards and contributing to agricultural and economic food production sustainability. Further, an organized means of compiling, archiving and disseminating information on these resources, knowledge, and cooperative efforts is necessary. The objective of GlobalFAMS is to further promote and advance all sectors of agricultural meteorology by improving international, inter-Society cooperation and partnership, exchange of knowledge and education in all the related scientific, technological, environmental, social issues.

GlobalFAMS may hence act as coordinating body of joint initiatives, promoted by its National member organizations, and as a synergy activator, and will have the purpose to promote the application of climate and weather information to agricultural production to reinforce global sustainable agriculture. GlobalFAMS is also planned to promote the exchange of information, tools and experience, the development of knowledge in agrometeorology and agroclimatology in order to support the growth, and promote current awareness about more efficient use of natural resources and the reduction/mitigation of weather/climate related impacts and hazards by early warning systems for producers and managers.

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