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Research article

Effect of foliar application of N and humic acids on growth and yield of durum wheat

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Abstract – The aim of this study was to monitor the effect of foliar application of humic acid on plant growth, photosynthetic metabolism and grain quality of durum wheat grown in a Mediterranean-type climate. Four fertilization treatments were applied: a non-fertilized control, a crop fertilized with foliar application of humic acid, a crop fertilized with mineral N on soil at sowing, tillering and stem elongation, and a crop fertilized with foliar application of N (ammonium-nitrate solution). Aboveground plant mass accumulation was measured throughout two growing seasons, and grain quality parameters were tested at harvest time. Gas exchange, leaf protein content and Rubisco activity were monitored at different stages of plant development. Differences between years were often relevant due to weather conditions. The foliar application of humic acid caused a transitional production of plant dry mass with respect to unfertilized control and split soil N application. This effect was also evident for grain yield, spike fertility and grain protein content during the two years of the study. Humic acid never affected photosynthesis or stomatal conductance, while Rubisco activity and leaf protein content showed intermediate responses between unfertilized control and split soil N application. We conclude that humic acid had limited promoting effects on plant growth, grain yield and quality, and photosynthetic metabolism of durum wheat crops grown in a typical Mediterranean-type agro-ecosystem of southern Italy, with respect to split soil N application.

humic acid / durum wheat / gas exchange / nitrogen / protein / Rubisco / grain quality

1. INTRODUCTION

The potential yield under non-limiting water and nutrient supply of a wheat crop is a function of the amount of light energy intercepted and utilized in carbon assimilation through photosynthetic processes (Araus et al., 1993), and the partitioning of carbon between aboveground mass and the grain (Evans and Fisher, 1999). Nitrogen supply to the plant will influence the amount of protein, protoplasm and chlorophyll formed. In turn, this influences cell size, leaf area and photosynthetic activity. Nitrogen availability plays a key role in determining tiller number, kernel number and kernel size in wheat plants. The rate of nutrients required by wheat plants varies depending on the dynamics of the crop-soil-weather interactions during the growth cycle (DeLacy et al., 1996). However, the largest amounts are adsorbed actively during the vegetative early stages of growth (McMaster, 1997). Under soil water deficit conditions, nitrogen N uptake by roots is limited and leaves of N-deprived plants have fewer and smaller cells (Fricke et al., 1997). The relative effect of varying rates of N delivery to the growing processes depends on the stage of plant development (Roggatz et al., 1999).

Variation in N management strategies may affect physiological traits, leading to significant quantitative and qualitative differences in grain yield. However, in Mediterranean-type agro-ecosystems crop yields may not be proportional to N input, because of the interaction between N-induced growth and its effect on water use and water availability for grain filling (Asseng et al., 2002). The goal of using nutrients efficiently in wheat crops, nowadays, is the primary objective of agriculture, because of the detrimental environmental effects that are associated with improper N management and its excessive use (Van de Geijn et al., 1994). The impact of agricultural practices on processes involved in N-use efficiency (Huggins and Pan, 1993) can be used to identify inefficiencies in management. Matching N supply with crop demand is important to optimize fertilizer uptake, and utilization and retention in the cropping system. Adequate N fertilization is a prerequisite to produce high yields of wheat and to increase grain quality, though depending on the background fertility level. High levels of protein are important for superior durum wheat flour (Feil, 1997).

Recently, among the fertilization strategies, the foliar spray with different molecules as humic acid has been introduced. These organic substances have no harmful threat to the quality

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of the environment (Senn, 1991). Under water stress, foliar fertilization with humic molecules increased leaf water retention and the photosynthetic and antioxidant metabolism (Fu Jiu et al., 1995). Foliar spray with humic acid also increased root length (Malik and Azam, 1985) and leaf area index (Figliolia et al., 1994). Research studies showed that humic acid can be used as a growth regulator to regulate hormone level, improve plant growth and enhance stress tolerance (Piccolo et al., 1992). O'Donnell (1973) found that humic acid from leonardite (a naturally occurring, highly compressed and decomposed, soft brown and coal-like organic material, usually found in conjunction with deposit of lignite) exhibits auxin-like effects. Tan and Nopamornbodi (1979) indicated that humic acid was in general beneficial to shoot and root growth of corn plants. In addition, the presence of humic molecules raised the effect on plants of the fertilization based on N, phosphorus and potassium (Pollhamer, 1993). In semiarid conditions, foliar application with humic acid may represent an alternative to conventional soil fertilization and a prompt source of N at grain filling. At grain filling, soil drying induced early senescence, reduced photosynthesis and shortened the grain-filling period in wheat plants (Johnson and Moss, 1976; Yang et al., 2000). Humic acid can stimulate shoot and root growth, and improve resistance to environmental stress in plants (Goatley and Schmidt, 1990), but the physiological mechanism has not been well established. We hypothesized that foliar applications with humic acid ameliorate leaf photosynthetic capacity and grain protein production of durum wheat in a Mediterranean-type agro-ecosystem.

The increasing variability of rainfall events and their distribution in Mediterranean environments (IPCC, 2001) might result in uneven responses to supplementary N fertilizer. Dry farming of high-protein durum wheat, therefore, carries a risk of over-fertilization, leading to substantial economic loss. The aim of this work was to study the effect of foliar application of fertilizer containing humic acid on growth, photosynthetic metabolism and grain protein content of durum wheat in Italy.

2. MATERIALS AND METHODS

The study was conducted in the 1997–1998 and 1998–1999 growing seasons at an experimental field site near Campobasso (Italy, latitude 35° 6' N, longitude 13° 10' E, altitude 621 m a.s.l.). Before starting the experiments, soil properties were analyzed according to standard procedures (SISS, 1985). The soil is characterized by a clay-sand texture (Tab. I) and rather large values of organic matter (though cation exchange capacity was not very high); its profile is uniform but contains low amount of nutrients (N, P and K), soil pH is relatively high (alkaline) but the salinity is average. The area has a typical mountainous Mediterranean climate of interior lands in southern Italy. A conventional meteorological station placed in the field site recorded climatic data. The previous crop for each experimental field was *Vicia faba* minor. Wheat (*Triticum durum* L.; cv. Duilio) was sown at a rate of about 350 seeds m^{-2}. Weeds were controlled by a combination of 2,4 D (2,4 dichlorophenoxy) and manual weeding during the crop cycle.

In each growing season, the treatments were a non-fertilized control, a crop fertilized with foliar application of humic extracts, a crop fertilized with N (as NH_4NO_3) applied on soil at sowing, tillering and stem elongation, and a crop fertilized with foliar application of N (as NH_4NO_3 solution). After ploughing (30 cm depth), the soil was given 44 kg ha^{-1} P as P_2O_5 and harrowed prior to sowing. Each treatment was replicated three times in a fully randomized block design (unit plot 10 m^2). The plots fertilized with N applied on soil received three dressings with 40 kg ha^{-1} of N each. In the first year N application on soil shifted the phenological stage by about five days. The main phenological growth stages for the two growing seasons were described following McMaster (1997):

Phenological phase	1997–1998	1998–1999
Emergence	15 December	18 December
Tillering	7 April	10 April
Anthesis	21 May	20 May
Grain filling	10 June	30 May
Harvest	10 July	12 July.

The foliar treatment was carried out with liquid solution at 12.6 mg L^{-1} of ammonium nitrate in deionized water and humic acid solution (a watery mixture of humic acid extracted from leonardite, oxidized lignite, with 12% of total dry matter on a fresh mass basis, 60% of total dry matter on a dry mass basis, 7% of N on a dry mass basis, undetectable oligo-elements, pH 7.5, C/N ratio 20) at 0.15% fresh weight in deionized water to reach the same N content of the ammonium nitrate solution.

These two solutions were sprayed on top of the leaves weekly for five treatments, starting from the beginning of stem elongation until grain filling, when the flag leaf was green and showed a consistent photosynthetic activity. Application of deionized water was sprayed on leaves of other treatments for uniformity. Differences in the amount of N between soil and foliar treatments were due to specific application features.

Table I. Particle size distribution and physical-chemical properties of the soil at the two experimental field sites (Campobasso, Italy).

	1998 Colle delle Api	1999 Polese
Coarse sand (%)	2.9	3.4
Fine sand (%)	33.1	34.1
Silt (%)	24.6	22.5
Clay (%)	39.4	40
pH (in water)	7.97	8.01
Total $CaCO_3$ (%)	12.5	12.2
Organic matter (%)	1.79	1.84
Cation exchange capacity (mq/100 g)	13.9	14.11
Active $CaCO_3$ (%)	1.3	1.4
Total N (‰)	0.16	0.17
Electrical conductivity (µS m^{-1})	396	402
Exchangeable K (ppm)	149	152
Assimilable P (ppm)	23	24

Morphological traits were recorded on each of three replicate plots. Whole plant dry mass accumulation was periodically determined on 15 replicate plants for each data point, throughout the stages of the crop cycle. Plants were oven-dried at 75 °C until constant weight and the dry mass measured. At harvest time grain yield and quality components were determined, including the percentage of humidity in kernels, the test weight (estimated by the GAC II method), the weight of 1000 seeds (by means of the handing procedure), the number of kernels per spike and of spikes m^{-2}, and the grain protein content. The protein content in kernels was determined by combustion with an automatic system (LECO FP 428), following the Dumas method.

At tillering (third decade of April), anthesis (mid-May) and grain filling (first decade of June), gas exchange was measured in the field with a portable gas analysis system (Li-6400, Li-Cor Inc., Nebraska, USA) equipped with a 6-cm^2 cuvette (Delfine et al., 2001). Photosynthetic rate and stomatal conductance were measured on fully expanded leaves, exposed to the same actual incident radiation and with the same surface temperature (across midday, at saturating PAR of 1500–2000 $\mu mol\ m^{-2}\ s^{-1}$). Each measurement was replicated on 10 plants, randomly sampled between treatments to avoid diurnal effects on PSII and experiencing similar field conditions of light and vapor pressure deficit.

Leaf discs (3.8 cm^2) were periodically (tillering, heading and grain filling) sampled from ontogenetically similar leaves of 10 replicate plants per treatment and frozen in liquid N immediately after gas exchange measurements. The determination of Rubisco (ribulose 1,5 diphosphate carboxylase-oxygenase) activity in wheat leaves was carried out as in Delfine et al. (1998). Briefly, frozen leaves were ground in a chilled mortar with 30 mg polyvinylpyrrolidone (PVPP), quartz sand, 2 mL of extraction buffer (100 mM bicine pH 8, 10 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 1 mM ethylendiamminetetracetic acid (EDTA), and 0.02% (w/v) bovine serum albumin (BSA). The solution was centrifuged at 10 000 *g* for 10 s. The supernatant was used to determine radiometrically the total carboxylase activity of Rubisco (Di Marco and Tricoli, 1983). Soluble protein content of the same frozen leaves was determined following the Bradford method.

Data were averaged on a plant basis and statistical analysis was conducted with the GLZ (Generalized Linear Models) procedures of Statistica (StatSoft Inc., Tulsa, Oklahoma, USA). ANOVA procedures were used to test for treatment effects on individual sampling dates. Treatment means were compared with the least-squares means procedure. Statistical comparisons were considered significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Weather conditions differed between the two seasons (Fig. 1). The total amount of precipitation was higher in the first growing season and relatively well distributed throughout the experimental period (October 1997–July 1998, 995 mm); conversely, the second growing season showed much less late spring rainfall (October 1998–July 1999, 710 mm). Differences in temperature between seasons were less pronounced, being

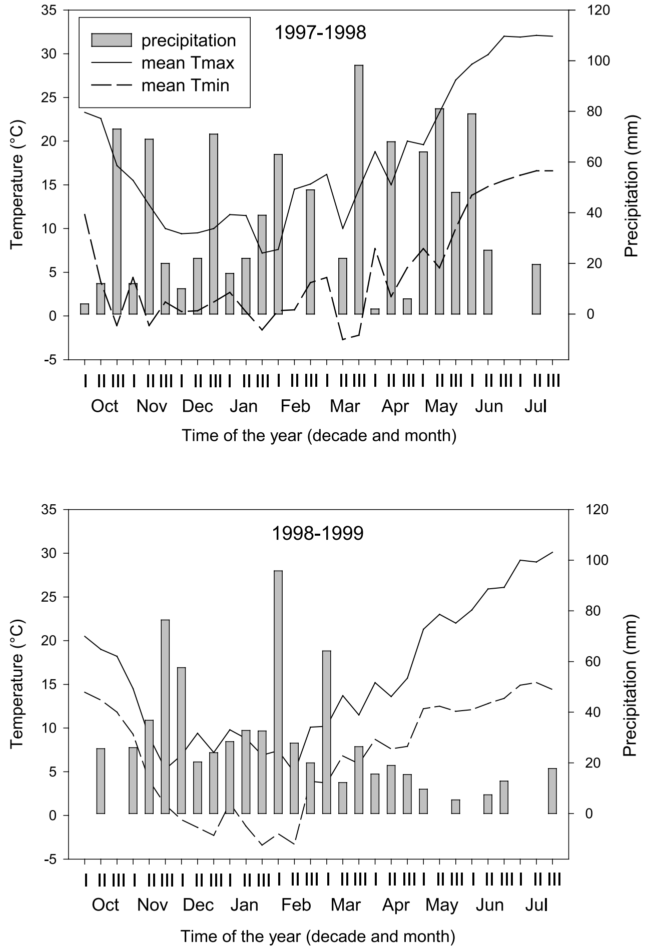

Figure 1. Total rainfall for each month decade, and averaged maximum (Tmax) and minimum (Tmin) temperatures as recorded by a field meteorological station near Campobasso (Italy).

nevertheless higher the averaged maximum temperature and lower the averaged minimum temperature in the 1997/98 than in the 1998/99 growing season (18.3 versus 15.6 °C, 5.3 versus 6.8 °C, respectively).

Plant dry mass accumulation was overall significantly higher in the first growing season than in the second (Fig. 2), starting from heading, but with similar seasonal trends. Differences among treatments, again, appeared first at heading and, starting from spikelet initiation, crops were ranked in three significantly different groups: non-fertilized control plants (averaging 3.4 and 2.9 g DW $plant^{-1}$ across treatments at physiological maturity, respectively, in 1998 and 1999), plants fertilized with foliar application of humic extracts and of N (averaging 4.2 and 3.3 g DW $plant^{-1}$ across treatments at physiological maturity, respectively, in 1998 and 1999), and plants fertilized with N on soil at sowing, tillering and stem elongation (averaging 4.8 and 3.6 g DW $plant^{-1}$ across treatments at physiological maturity, respectively, in 1998 and 1999).

Grain yield was significantly higher in the 1998 than in the 1999 harvest (25–33%), regardless of the treatment (Tab. II).

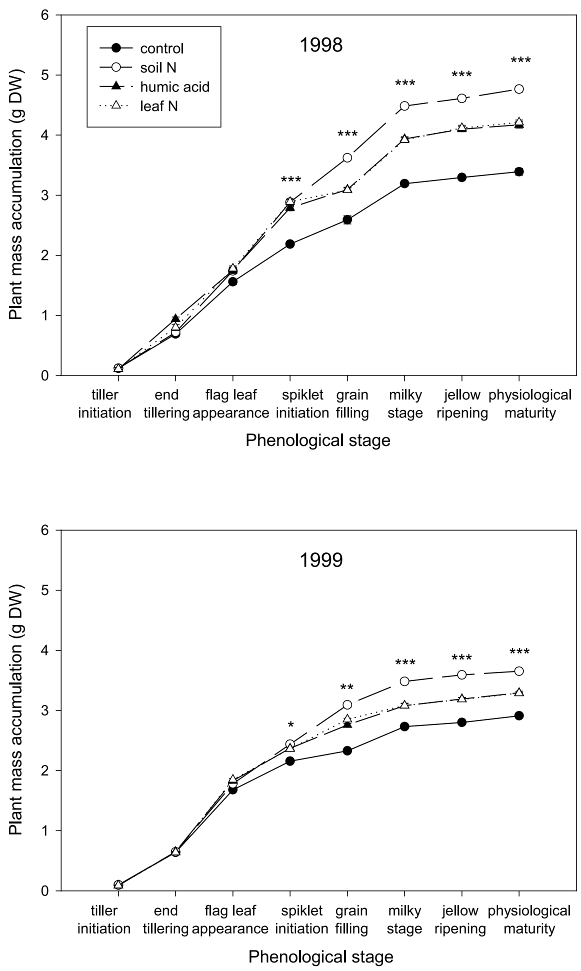

Figure 2. Accumulation of plant mass (dry weight, DW) in durum wheat plants at different phenological stages, subjected to four different fertilization treatments. Vertical bars represent the mean ± one standard error of the mean; $n = 15$ replicates. Treatments are referred to by symbols in the legend. Significant differences on a particular date are indicated as * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; significance for differences between years is reported in the text.

The number of kernels per spike was the same in both years (about 25) and the number of spikes m^{-2} behaved similarly (averaging 320 and 322 across treatments, respectively, in the 1998 and 1999 harvests). For these three parameters, ranking of treatments was similar in both years, values being consistently higher in plants fertilized with N on soil at sowing, tillering and stem elongation, intermediate in plants fertilized with foliar application of humic extracts and of N, and lower in non-fertilized control plants. The percentage of humidity in kernels did not differ between years and among treatments (averaging 11.7%, pooled data) (Tab. II). The test weight was higher in the 1998 than in the 1999 harvest (ranging from 8–9%) and likewise for the weight of 1000 seeds (ranging from 37–39%), regardless of treatment (Tab. II); differences among treatments were never significant, but there was a tendency for a lower weight of 1000 seeds in treatments fertilized with N on soil at sowing, tillering and stem elongation. The grain protein content was markedly higher in the 1999 than in the 1998 harvest (ranging from 5–8%), regardless of treatment (Tab. II); ranking of treatments was similar in both years, values being significantly higher in plants fertilized with N on soil at sowing, tillering and stem elongation, intermediate in plants fertilized with foliar application of humic extracts and of N, and relatively lower in non-fertilized control plants.

Gas exchange measured at tillering, anthesis and grain filling did not differ significantly among treatments (Fig. 3). Stomatal conductance and photosynthetic rate increased consistently from tillering to anthesis, then decreased significantly at grain filling; differences in gas exchange between years were of minor importance at tillering and heading, but a strong and significant reduction was observed at grain filling in 1999 as compared with 1998 (averaging 35 and 28% across treatments, respectively, for stomatal conductance and photosynthetic rate).

Soluble protein content in leaves decreased significantly from tillering to heading and grain filling, regardless of treatment (Fig. 4), such a trend being more evident in 1998 than in 1999. Protein content did not differ significantly between years, averaging 7.3 g m^{-2} across treatments and years. Differences among treatments were significant at heading and grain filling, but not at tillering, in both years; ranking of treatments was similar in both years, values being markedly higher in plants fertilized with N on soil at sowing, tillering and stem elongation, intermediate in plants fertilized with foliar application of humic extracts and of N, and lower in non-fertilized control plants. Similarly, Rubisco activity in leaves decreased consistently from tillering to heading and grain filling, regardless of treatment (Fig. 4). Rubisco activity did not differ consistently between years, averaging 76.3 μmol m^{-2} s^{-1} across treatments and years. Again, differences among treatments were significant at heading and grain filling, but not at tillering, in both years; ranking of treatments was similar in both years, values being significantly higher in plants fertilized with N on soil at sowing, tillering and stem elongation, followed by plants fertilized with foliar application of humic extracts and of N, and non-fertilized control plants.

Nitrogen plays a key role in plant nutrition. This is the mineral element required in the greatest quantity by cereal crop plants and it is the nutrient most often deficient. As a result of its critical role and low supply the management of N resources is an extremely important aspect of crop production (Novoa and Loomis, 1981). Production varies greatly from season to season, also caused by rainfall variability. Wheat crops respond to water deficit through changes in various physiological and metabolic processes (Chandrasekar et al., 2000). The lack of rainfall (spring 1999) was reflected in lower plant mass accumulation and less differences between treatments. The kernel weight under soil drying conditions may be reduced, since the loss of photosynthesis does not compensate for the gain from increased remobilization of carbon reserves (Yang et al., 2000). When there was sufficient rain (spring 1998) to ensure little or no water stress, the fertilized crops had a higher yield potential due to the vigorous vegetative growth stimulated by the higher N availability for soil (in particular in the split treatment) and foliage applications, resulting in additional spikes produced, and could fill the grain from photosynthesis as well as from remobilized sugars during the periods of peak demand (Yang et al., 2001). The low N crop experienced relatively less drought

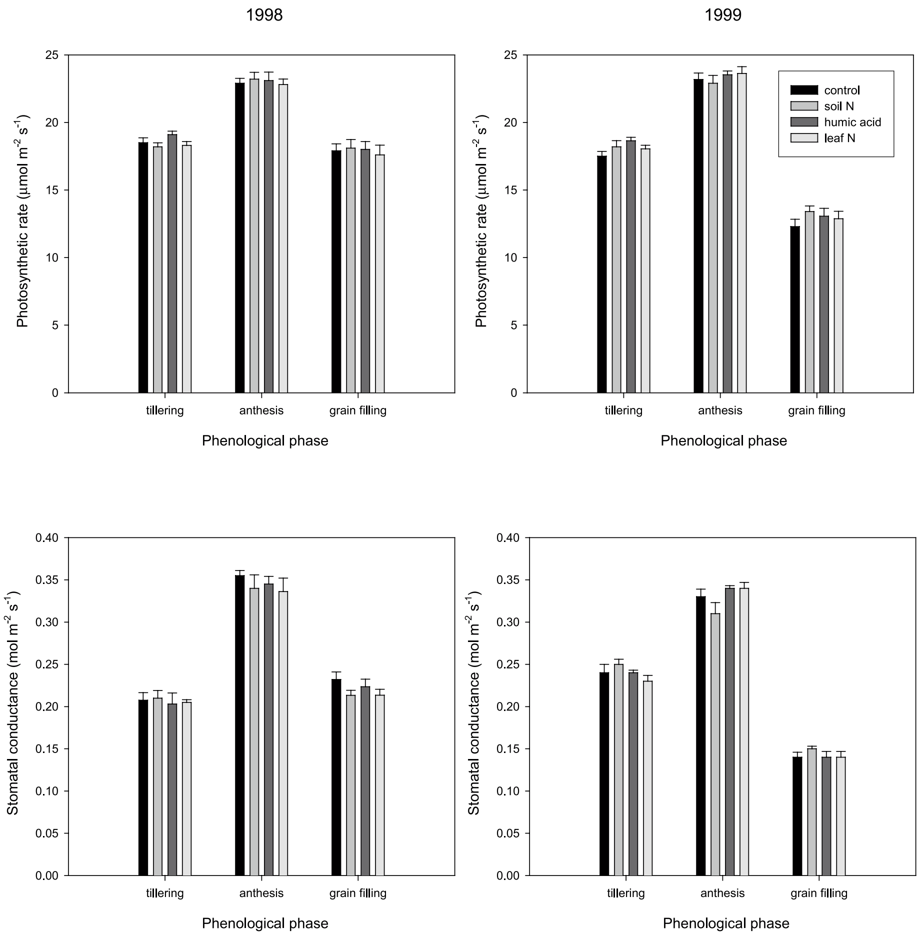

Figure 3. Changes in gas exchange (net photosynthesis and stomatal conductance) in durum wheat plants at different phenological phases, subjected to four different fertilization treatments. Vertical bars represent ± one standard error of the mean; $n = 10$ replicates. Treatments are referred to by symbols in the legend. Mean values in the same phenological phase and year were never significantly different according to the LSD test; significance for differences between years is reported in the text.

Table II. Grain yield and quality components at harvest time (1998 and 1999) for durum wheat grown nearby Campobasso (Italy) and subjected to four different fertilization treatments. Data are the means (± one standard deviation, not reported for humidity; $n = 15$ replicates). Mean values in the same column and year followed by the same letter are not significantly different ($P > 0.05$) according to LSD-test; significance for differences between years is reported in the text.

1998

Treatment	Grain yield (t/ha)	Humidity (%)	Test weight (kg/hL)	1000-seed weight (g)	Kernel/spike (number)	Spikes/m^2 (number)	Protein content (% DW)
Control	2.93 ± 0.2 a	11.6 a	81.9 ± 0.6 a	48.3 ± 0.7 a	20 ± 1.7 a	300 ± 7 a	13.4 ± 0.3 a
Soil N	4.61 ± 0.1 b	11.7 a	82.3 ± 0.8 a	46.3 ± 0.7 a	30 ± 2.0 b	340 ± 10 b	16.5 ± 0.3 b
Humic acid	3.59 ± 0.2 c	11.5 a	81.5 ± 0.5 a	48.1 ± 0.8 a	24 ± 1.1 c	319 ± 8 c	14.4 ± 0.3 a
Leaf N	3.58 ± 0.1 c	11.9 a	81.8 ± 0.4 a	48.3 ± 0.9 a	25 ± 1.9 c	321 ± 9 c	14.5 ± 0.4 a

1999

Treatment	Grain yield (t/ha)	Humidity (%)	Test weight (kg/hL)	1000-seed weight (g)	Kernel/spike (number)	Spikes/m^2 (number)	Protein content (% DW)
Control	2.21 ± 0.2 a	11.7 a	75.9 ± 0.6 a	35.1 ± 0.7 a	19 ± 1.9 a	299 ± 9 a	14.5 ± 0.3 a
Soil N	3.71 ± 0.2 b	11.5 a	75.8 ± 0.5 a	33.4 ± 1.0 a	31 ± 1.5 b	349 ± 9 b	17.4 ± 0.5 b
Humic acid	2.79 ± 0.2 c	11.8 a	74.5 ± 0.7 a	35.1 ± 0.8 a	25 ± 2.0 c	321 ± 8 c	15.5 ± 0.3 a
Leaf N	2.81 ± 0.1 c	11.8 a	75.3 ± 0.4 a	35.3 ± 0.9 a	24 ± 1.8 c	320 ± 10 c	15.6 ± 0.4 a

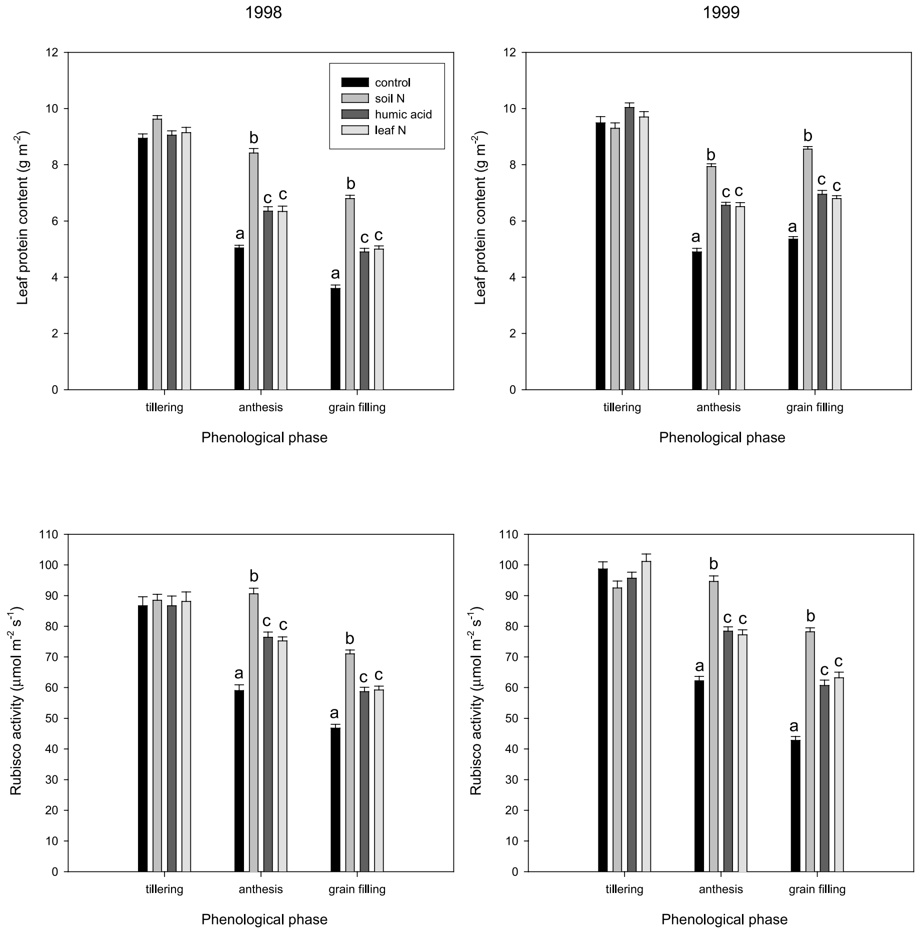

Figure 4. Changes in leaf protein content and Rubisco activity in durum wheat plants at different phenological phases, subjected to four different fertilization treatments. Vertical bars represent ± one standard error of the mean; $n = 10$ replicates. Treatments are referred to by symbols in the legend. Mean values in the same phenological phase and year marked with different letters (when present) are significantly different ($P \leq 0.05$) according to the LSD test; significance for differences between years is reported in the text.

stress during grain filling (1999 vs. 1998) than high N crops because of less growth to flowering, resulting in lower water use. In this sense, delaying application of some N until later in the season or splitting fertilization treatments would not necessarily limit the yield potential, allowing a more informed decision based on the soil water storage, crop N test and seasonal outlook. Humic acid-treated wheat plants showed somewhat positive growth responses on this relatively poor soil, while humic acid may have limited growth-promoting effects on plants adequately supplied with nutrients (Cooper et al., 1998).

The yield performance of the wheat plants indicated the weakness of distributing N through foliar application (regardless of the method) as compared with split applications of soil N. Differences in yield among treatments resulted mainly from the diverse number of kernels per spike and spikes per m^2, and secondarily from the weight of 1000 seeds. Differences in yield between years were mostly due to the smaller size of kernels (1999 vs. 1998), thus detrimental weather conditions did not alter patterns of grain yield; drought stress appeared only after anthesis, at grain filling. In fact, plant dry matter was the same across treatments until heading, when spikes differed in dry weight. Grain filling and partitioning of assimilates to developing grains are usually affected by post-anthesis water deficit (Broklehurst, 1978; Kobata et al., 1992; Palta et al., 1994). Plants amended with split applications of soil N showed relatively lower 1000-seed weight, probably for the greater number of kernels per spike with respect to other treatments. In alkaline soils, as in the present case, use of humic acid has been found to increase wheat yield by 25% (Wang et al., 1995), which is a value similar to our findings (23 and 26% compared with control, respectively, for 1998 and 1999). Spraying with fulvic acid has been found to enhance the yield of wheat grown under dry conditions (Xudan, 1986).

On soils that originally contain low levels of N, as in the present case, the applied N, if insufficient, is utilized primarily for vegetative growth and there is not enough left for maximum protein production. Experimental N treatments allowed the increase in grain protein content up to a maximum of almost 18% (reached with split soil N application), while maintaining yield; but in the experiment's range of about a 30% increase in protein, yield gain was still about 50% (correlation between grain yield and protein content across treatments was strongly significant; $P < 0.0001$, $r^2 = 0.99$, each year, data not shown). The effects of split application of N tend to be variable, and frequent negative correlations between protein and yield are found (Feil, 1997); as yield increases, protein content decreases due to dilution effects. In this experiment, foliar applications with liquid N, either as humic acid or ammonium nitrate, were not successful in increasing grain yield and protein content of durum wheat as compared with fertilization with N on soil at sowing, tillering and stem elongation.

The relative efficiency of N fertilizers as affected by time of application and method of placement varies greatly from year to year due to environmental conditions (Milford et al., 1993). A positive effect of humic acid might be expected if applied on durum wheat leaves in periods characterized by marked water shortage, particularly in the final stages of the crop cycle. Across treatments, there was a continuous progress in N-use efficiency as measured by the protein yield of the durum wheat, further demonstrating the advantage of split applications of soil N for quality expression. Indeed, crop N utilization depends on total N supply as well as on its distribution scheme (Sticksel et al., 1999). Without water stress, N fertilization is necessary for quality attributes to be expressed, particularly gluten quality. In contrast, under drought conditions, N fertilization may not be so important to realize gluten quality. However, kernel protein content increased with N fertilization, also in the second year of experiment. Durum wheat in Mediterranean environments copes with fast-developing drought conditions during the last part of its cycle. Our experimental site was located in a mountainous inland region with cold rainy winters and cool springs, which may have buffered somewhat the markedly negative effects of early summer drought on plant performance and grain quality. The relatively drier weather conditions in 1999 may have favored an average greater translocation efficiency of N from the vegetative plant parts after anthesis to the grain (Campbell et al., 1983; McNeal et al., 1968), and reduced final grain yield as a result of a faster senescence of the photosynthetic organs (Nicolas et al., 1984).

The negative correlation between kernel number and kernel weight indicated a lack of feedback between the sink and photosynthetic rates (Asseng et al., 2002). The relative stability of photosynthetic components and high photochemical efficiency may help to maintain photosynthesis at grain filling in water-stressed wheat plants (Martinez et al., 2003). However, quantum yield of PSII electron transport may be reduced in field-grown wheat plants adapted to high irradiance under drought conditions (Lu and Zhang, 1999). The durum wheat plants of this experiment showed a reduction in relative values of gas exchange at grain filling during the relatively drier year. Water deficit at both the vegetative and the anthesis stage has been shown to significantly reduce photosynthetic rates (Brar et al., 1990). Adaptive mechanisms to drought of plants may result in varying relationships between foliar gas exchange, seasonal pattern and crop productivity (Jones and Corlett, 1992). Humic acid influences respiration and photosynthesis, formation of complex with mineral ions, catalysis to enzymes, and stimulation of nucleic acid metabolism (Schnitzer and Khan, 1972). The photosynthetic rate of plants treated with humic acid has been shown to increase, though the effect of these substances on photosynthetic pigments, N and other nutrients is controversial (Liu et al., 1998). This makes the relationship between gas exchange and crop yield in durum wheat difficult to interprete.

The way in which the pooled assimilation rate and stomatal conductance of durum wheat were correlated was best described by a curvilinear correlation or by a linear relation not passing through the origin ($P < 0.0001$, $r^2 = 0.95$ or 0.93, respectively, data not shown), which indicates that either the assimilation rate or conductance responded more strongly than the other parameter to changes in environmental or plant internal conditions. In this case, water-use efficiency is not maintained constant, increasing progressively with decreasing soil water availability (Turner, 1997). Indeed, intercellular CO_2 concentration decreased, as stomatal conductance was more affected by changes in environmental or plant internal conditions than the assimilation rate. A weak relationship between photosynthetic rate and stomatal conductance, implying that non-stomatal limitations to photosynthesis play a major role, may be expected under drought stress (Siddique et al., 1999). Nabati (1991) reported that humic acid enhanced drought tolerance of Kentucky bluegrass. Nitrogen deficiencies usually result in lower levels of photosynthetic enzymes per unit area, though durum wheat plants showed a lack of correlation between Rubisco activity or soluble protein content and the photosynthetic rate of leaves.

As the canopy developed and older tissue contributed more than the total tissue in terms of N, Rubisco activity and soluble protein content in durum wheat leaves decreased with the season, though plant dry mass increased (Wilhelm et al., 2002). Maximum Rubisco activity was affected positively by N fertilization. It has been suggested that Rubisco could be used as an early selection criterion for high crop yield (Murthy and Singh, 1979). However, Rubisco levels may be affected by environmental conditions in successive developmental stages (Martinez et al., 2003). Foliage Rubisco activity and soluble protein content depicted different yield performances between treatments better than traditional gas exchange. Humic acid had a positive effect on photosynthetic enzymes of durum wheat. Humic acid has been found to alter micronutrient uptake in wheat plants, being effective at ameliorating leaf interveinal chlorosis (Mackowiak et al., 2001).

4. CONCLUSION

Given the higher number of foliar treatments required and the associated costs, and on the basis of the experimental evidence, the use of humic substances on a larger scale does not appear nowadays advisable for sustainable durum wheat production in southern Italy; though a relatively lower cost of the application of these products by foliar spray compared with soil applications has been indicated (Chen and Aviad, 1990). However, results obtained for plant and yield performances raise questions on the use of these products in sustainable agriculture, particularly taking into account the target for a higher nutrient-use efficiency of durum wheat crops. Our data indicate that foliar application of humic substances do not improve consistently the nutritional status of durum wheat and, therefore, do not compensate for the imbalance of mineral nutrition. New research to examine the viability of applying humic acid at different growth stages and in the presence of soil N fertilization is needed, considering that excessive rates can cause tissue burning and crop injury. Gas exchange during kernel filling appeared to vary with phenological stage and soil moisture availability, but less with N supply. The development of management strategies to enhance N-use efficiency and the identification of suitable genotypes to fit this new approach for sustainable agriculture in Mediterranean agro-ecosystems are warranted.

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