European Journal of Agronomy

THE OFFICIAL JOURNAL OF THE
EUROPEAN SOCIETY FOR AGRONOMY

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As durum wheat productivity is affected by nitrogen fertilisation management in Central Italy

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ARTICLE INFO

ABSTRACT

Optimisation of N fertilisation is a central issue and goal of applied research in agricultural systems. Site-specific management techniques are needed in order to closely match availability with requirement throughout crop cycle and to reduce as much as possible environmental dispersion of N. Lysimeter experiments were conducted in central Italy in two subsequent seasons to investigate the response of two commercial durum wheat cultivars to different N fertilisers applied before seeding and at topdressing, and to split applications of N. Grain yield and yield components, N uptake and N leaching were determined. Ammonium sulphate (AS) and urea containing the nitrification inhibitor 3,4-dimetihyl pyrazole phosphate (Entec® 46) were applied before seeding; AS, ammonium nitrate sulphate containing the nitrification inhibitor 3,4-dimetihyl pyrazole phosphate (Entec® 26), and urea were applied at 5th leaf unfolded stage. Six N-fertiliser treatments with a total N amount of 180 kg N ha^{-1} were tested, consisting of splitting application before seeding, at GS15 and GS30 respectively of 0-180-0, 0-90-90, 30-150-0, 30-75-75, 60-120-0 and 60-60-60 kg N ha^{-1}. In both years, fertiliser splitting affected durum wheat grain yield and N concentration; variations due to splitting reached 1 t in grain yield and 7 g kg^{-1} in N concentration of grain, corresponding to 4% increase in protein content. Highest grain yield, protein concentration, nitrogen use efficiency (NUE), and nitrogen uptake efficiency (NUpE) were obtained with the application of 30 kg N ha^{-1} before seeding. The yield advantage was related to higher number of kernels per spike, resulting from higher number of fertile spikelets per spike. Grain yield was not affected by nitrogen source applied before seeding, but was modified by topdressing N fertiliser. Yield increased by 0.4 t ha^{-1} with urea, compared to AS and Entec® 26, owing to a greater number of kernels per spike. Nitrogen leaching was closely related to rainfall: total amount of N lost during wheat cycle was almost entirely accounted for N leaching in winter, before topdressing N application. As a consequence, the quantity of N lost by leaching increased with the increase of N rate applied before seeding, while topdressing fertiliser did not affect losses.

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1. Introduction

Optimisation of mineral N fertilisation aimed to increase N use efficiency is a central issue and goal of applied research in agricultural systems. Nitrogen use efficiency of worldwide cereal crops was estimated to be near 33% (Raun and Johnson, 1999), ranging from 14 to 59% in wheat (Melaj et al., 2003; López-Bellido et al., 2005); which suggests that current N strategies are extremely inefficient.

Nitrogen use efficiency (NUE) in cereal grain production may be low owing to losses of N by volatilisation, denitrification and leaching. Ammonium-based nitrogen fertiliser products are susceptible to volatilisation losses of nitrogen if surface-applied and not incorporated. Bouwman et al. (2002) estimated that NH$_3$ volatilisation losses from synthetic N fertilisers applied to both winter and summer crops amounts to 7% in industrialised countries. In Europe, fertiliser N losses via denitrification were estimated at 0.2–2.9% of the N applied to cereals (Kaiser et al., 1996).

Leaching losses occur when rainfall exceeds crop evapotranspiration and downward water movement through the soil profile takes place, which corresponds to the fall-winter period in humid Mediterranean climate (Arregui and Quemada, 2006). Leaching N–NO$_3$ losses from durum wheat crop were estimated in central Italy at 21–32 kg N ha^{-1} yr^{-1}, corresponding to 12–22% of the total N applied (Ercoli et al., 2012). Nitrate leaching in a 3-year crop rotation wheat–barley–rapeseed in similar conditions in Spain was

estimated at 31–35 kg N ha^{-1} y^{-1}, or 25–29% of the total N applied (Arregui and Quemada, 2006). Thus, as a general rule, N leaching in Mediterranean climate can be regarded as the greatest source of N loss and the major determinant of low N utilisation efficiency.

Durum wheat is widely cultivated throughout the Mediterranean region, where the crop is typically planted in November and harvested at the beginning of July. Heavy rainfall and low crop evapotranspiration rates can be expected between November and March, resulting in higher risk of N leaching losses. To reduce leaching losses, fertiliser application should aim to match as much as possible the requirement of plant N with the available nitrogen in soil, minimising the excess of N in soil useless to the plant. Thus, application should be the latest possible compatible with the stage of development that still permits rapid N uptake, in order to reduce the opportunities for N losses of unused N (Raun et al., 2008).

A number of experiments in winter cereal have shown that split applications of N fertiliser involving equal quantities of N applied before and after seeding are agronomical strategies to improve N utilisation efficiency (Alcoz et al., 1993; Delogu et al., 1998; López-Bellido et al., 2005, 2012; Dilz, 1988). Sowers et al. (1994) and Strong (1995) found that the N fertiliser applied in autumn, owing to increased N leaching, had a lower efficiency than when the nitrogen was splitted in fall and spring. Garrido-Lestache et al. (2004) observed that timing and splitting of N fertiliser influenced grain protein content, which peaked when half or one-third of the N rate (150 kg N ha^{-1}) was applied at stem elongation, and in some cases when N was applied only at tillering. However, the effect of splitting N rate on grain quality was unclear in other studies (Ayoub et al., 1994; Garrido-Lestache et al., 2005). Fuertes-Mendizábal et al. (2010) found that the splitting of the same N rate in three amendments (stages GS20, GS30, and GS37, according to Zadoks scale) instead of two (stages GS20 and GS30) did not modify grain yield but improved grain protein content, indicating that a late N application may have more impact on N metabolism and N remobilisation than on biomass accumulation and grain yield. Ottman et al. (2000) showed higher grain protein contents with the application of high N rates at anthesis.

Another strategy to reduce N leaching losses and to increase N utilisation efficiency is the use of fertilisers releasing reduced and controlled amount of nitric N. Several studies have shown beneficial effects of the use of slow and controlled-release fertilisers, stabilised fertilisers and/or nitrification and urease inhibitors to enhance productivity of agricultural and horticultural crops (Prasad and Power, 1995; Pasda et al., 2001; Carreres et al., 2003). In other studies, however, no positive results have been reported following the application of fertilisers that delay nutrient availability (Cartagena et al., 1995; Diez et al., 1997; Arregui and Quemada, 2008). Inconsistencies in results may appear since soil properties and/or climatic conditions affect both nutrient-release rates from fertilisers and processes leading to leaching and denitrification losses and immobilisation in soil.

The present work was undertaken to determine the effects of different sources of N fertiliser distributed before seeding and topdressing and split applications of N on grain yield, yield components, N uptake and N leaching of durum wheat, and to identify N fertiliser and type of splitting that optimised N uptake and retention in Mediterranean environments.

2. Material and methods

Lysimeter experiments were conducted in two successive growing seasons, 2008–2009 and 2009–2010 at the experimental station of the Department of Scienze Agrarie, Alimentari e Agroambientali of the University of Pisa, Italy, that is located at a distance of approximately 10 km from the sea (43°40′ N, 10°19′ E) and 3 m above sea level. The climate of the site is cold, humid Mediterranean with mean annual maximum and minimum daily air temperatures of 20.2 and 9.5 °C, respectively (Moonen et al., 2001). For both years, daily weather data were obtained from a meteorological station located within 100 m from the experimental fields.

In both years a full factorial of two durum wheat varieties, two N fertilisers before sowing, three topdressing N fertilisers and six split applications of N was replicated two times and arranged in a randomised, complete-block design. The durum wheat (*Triticum durum* Desf.) varieties were Latinur and Svevo, currently used in local production. The two N fertilisers before sowing were ammonium sulphate (AS), and urea containing the nitrification inhibitor 3,4-dimetihyl pyrazole phosphate (Entec® 46). The three topdressing N fertilisers were AS, ammonium nitrate sulphate containing the nitrification inhibitor 3,4-dimetihyl pyrazole phosphate (Entec® 26), and urea, and were applied at the 5th leaf unfolded stage. Total N amount of 180 kg ha^{-1} was split among pre-seeding, at 5th leaf unfolded stage and at pseudo stem erection as follows: 0-180-0, 0-90-90, 30-150-0, 30-75-75, 60-120-0 and 60-60-60 kg N ha^{-1}. Growth stages of 5th leaf unfolded and pseudo stem erection were individuated following the scale of Zadoks (Zadoks et al., 1974), corresponding respectively to GS15 and GS30.

Open-air lysimeter installation consisted of 144 lysimeters of 0.50-m length, 0.50-m width and 0.4-m height (100-L volume), arranged in four rows of 36, spaced 20 cm and embedded in expanded clay to smooth daily fluctuations in soil temperature. Lysimeters were filled with a soil tamped to about original soil bulk density, and were attached to a 3-cm rigid PVC drain that ended in a central collection facility. Soil chemical–physical properties were: 68.5% sand (2 mm > Ø > 0.05 mm); 25.9% silt (0.05 m > Ø > 0.002 mm); 5.6% clay; 7.0 pH; 2.0% organic matter (Walkley and Black method); 0.8 g kg^{-1} total nitrogen (Kjeldahl method); 26.0 mg kg^{-1} available P (Olsen method); 76.3 mg kg^{-1} available K (BaCl$_2$ + TEA method).

Phosphorus and potassium were hand applied preplanting as triple mineral phosphate and potassium sulphate at a rate of 150 kg ha^{-1} P$_2$O$_5$ and 150 kg ha^{-1} K$_2$O. Nitrogen was hand applied before seeding and at GS15 according to the treatments and at GS30 as urea.

The durum wheat varieties, Latinur and Svevo, were sown on 18 November 2008 and 25 November 2009, within the optimum planting time for wheat production in Central Italy. A rate of 100 viable seeds per container, corresponding to 400 viable seeds m^{-2}, in rows spaced 15 cm apart, was applied. Plant emergence was recorded on 5 December 2008 and 9 December 2009. Weed control was performed throughout wheat cycle by hand hoeing.

Leachates from each lysimeter were collected during the entire research period in both years after each major rainfall event in a 20-L PVC tank. Leachate volumes were measured and their N–NO$_3$ concentrations were determined with an Orion ion analyser model 502A (Orion Research Inc., Boston, MA, USA). Accumulated monthly leachate volumes were calculated and the monthly flow-weighted N–NO$_3$ concentrations were determined by summing up N–NO$_3$ mass collected for each rainfall event in a month divided by the total leachate volume collected in the month. Averagely, a concentration of 2.4 mg N–NO$_3$ L^{-1} was determined in rainfall. For all treatments, timing of stage of 1st leaf emergence (GS10), 5th leaf unfolded (GS15), pseudo stem erection (GS30), inflorescence emergence (GS50), and physiological ripening (GS90) were recorded, expressing in thermal time the duration of the periods between stages (Table 1). Thermal time was calculated following McMaster and Wilhelm (1997), assuming 2 °C as base temperature (Porter and Gawith, 1999). At GS90 (22 June 2009 and 30 June 2010) plants from each container were manually cut at ground level and were partitioned into culms, leaves, chaff and grain. For dry weight

Table 1
Accumulated growing degree days and rainfall during the whole wheat cycle and main growth periods. Growth stages are defined following Zadok's scale (GS00 seeding, GS10 emergence, GS15 5th leaf unfolded stage, GS30 pseudo stem erection, GS50 anthesis, GS 90 physiological maturity).

Period	GDD (°C)		Rainfall (mm)	
	2008–2009	2009–2010	2008–2009	2009–2010
GS00–GS10	131.9	130.8	169.2	52.4
GS10–GS15	408.9	346.9	206.4	254.8
GS15–GS30	144.4	184.4	80.0	58.8
GS30–GS50	671.9	573.4	220.6	100.0
GS50–GS90	1048.7	1123.6	69.6	259.8
Whole cycle	2405.7	2358.9	745.8	725.8

determination, samples from all plant parts were oven dried at 65 °C up to constant weight. Mean kernel dry weight was also measured and number of kernels per unit area and harvest index (HI) were calculated. Samples of each plant part were analysed for nitrogen concentration (Kjeldahl method); N contents were calculated by multiplying N concentration by dry weight.

Nitrogen efficiency parameters were calculated for each treatment according to Moll et al. (1982) and López-Bellido et al. (2005). Nitrogen use efficiency (kg kg^{-1}) was the ratio of grain yield biomass to N fertiliser. Nitrogen uptake efficiency (NUpE, kg kg^{-1}) was the ratio of total aerial plant part N uptake to N fertiliser. Nitrogen utilisation efficiency (NUtE, kg kg^{-1}) was the ratio of grain yield biomass to total N uptake. Nitrogen harvest index (NHI) was the ratio of N in grain to total N uptake.

Results were treated by ANOVA. The main effects of year, variety, N fertiliser before seeding, N fertiliser topdressing, N splitting, and their interactions were tested for dry weight of plant parts and relative N concentration and content and total N leached. The main effects of variety, N fertiliser before seeding, N fertiliser topdressing, N splitting, and their interactions were tested for drainage water, N concentration and N leaching in the months from November to April in the two seasons. The CoHort software package ver. 6.4 (CoHort software, Monterey, CA, USA) was used. Significantly different means were separated at the 0.05 probability level by the least significant difference test (Steel et al., 1997).

3. Results

3.1. Weather conditions

Total rainfall during wheat growing cycle was similar in the 2008–2009 and 2009–2010 growing seasons (Fig. 1 and Table 1) and was by 42% higher compared to the mean rainfall for the area over the last 10 years (515 mm). Rainfall from seeding to GS30 was similar in both years, accounting for over 50% of total rainfall during wheat cycle, whereas it differed during jointing and grain filling. During jointing, rainfall was about 3-fold in 2008–2009 compared to 2009–2010, while during grain filling it was about 3-fold in 2009–2010 compared to 2008–2009. Temperature was lower than the long-term average in both seasons, as the thermal time up to maturity was 2574 °C in the long-term average, vs. 2406 °C in 2008–2009 and 2359 °C in 2009–2010 (Table 1). Differences between seasons in accumulated growing degree days were recorded only in the period from GS10 to GS50. The highest difference between seasons was recorded from GS15 to GS30, with 28% higher value in 2009–2010 compared to 2008–2009.

3.2. Grain yield and yield components

Grain yields were always higher in 2009–2010 crop season with all splittings. In both growing seasons the highest yield was
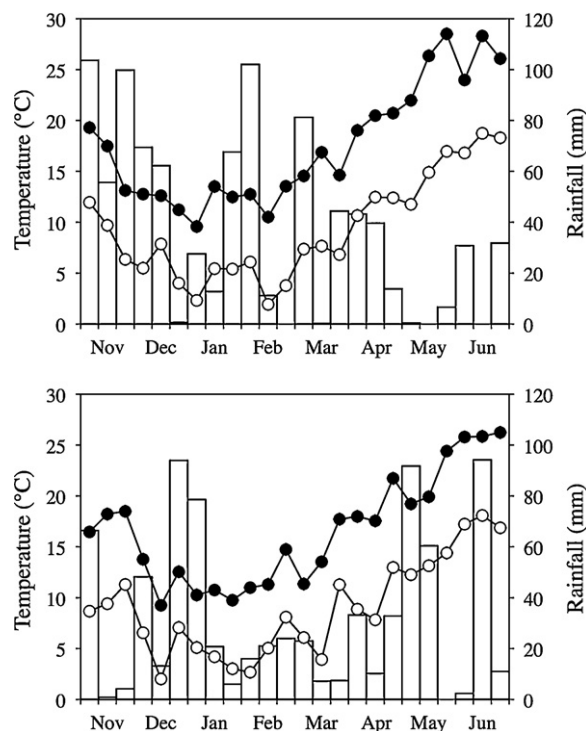

Fig. 1. Rainfall (bars), maximum (closed symbol) and minimum (open symbol) temperature in 2008–2009 (up) and 2009–2010 (down) seasons.

obtained with the 30-150-0 splitting, while the application of 0-180-0 in 2008–2009 and 60-120-0 in 2009–2010 resulted in the lowest grain yield (Fig. 2). Differences in grain yield between years and splittings were due to variations in number of spikes per unit area, number of kernels per spike and mean kernel weight. The average number of spikes m^{-2} was 559 in 2009–2010 season and 484 in 2008–2009 (results not shown) and was higher with 0-180-0 and lowest with 60-60-60 (Table 2). The number of kernels per spike was higher in 2009–2010 only for the applications without N before seeding (0-180-0 and 0-90-90), while for the other four splittings it was the same in the two growing seasons (Fig. 2). In both years the highest values were recorded with 30-150-0, 60-60-60 and 30-75-75 splittings, and lowest with 0-180-0. Maximum variations due to different splittings were 8.4 kernels per spike in 2008–2009 and 6.1 kernels per spike in 2009–2010. Mean kernel weight did not vary between years while the highest values among nitrogen splittings were obtained by 30-75-75, 60-60-60 and 0-90-90 treatments, and the lowest by 60-120-0 (Table 2). In summary, the highest grain yield obtained with the treatments 30-150-0 and 30-75-75 derived from the combination of low number of spikes, high number of kernels per spike and medium-high mean kernel weight. Conversely, the lowest grain yield recorded in the treatments 0-180-0 in 2008–2009 and 60-120-0 in 2009–2010 were due

Table 2
Harvest index, spike number, and mean kernel weight. Nitrogen splitting mean values.

Nitrogen splitting	Harvest index (%)	Spike number (n m^{-2})	Mean kernel weight (mg)
0-180-0	33.3 ab	595.6 d	40.3 b
0-90-90	37.2 c	549.5 c	41.4 c
30-150-0	35.8 bc	508.9 b	40.2 b
30-75-75	37.6 c	494.9 ab	41.8 c
60-120-0	31.9 a	509.4 b	38.9 a
60-60-60	36.3 bc	468.8 a	41.5 c

Within columns, numbers followed by the same letter are not significantly different at $P \leq 0.05$.

Table 3
Grain yield, vegetative dry weight, harvest index, spike number, kernel number and mean kernel weight. Variety and top dressing nitrogen fertilisers mean values.

Treatments	Grain yield (t ha^{-1})	Vegetative d.w. (t ha^{-1})	Harvest index (%)	Spike number (n m^{-2})	Kernel number (n spike^{-1})	Mean kernel weight (mg)
Variety						
Latinur	4.6 b	8.2 b	35.7 a	555.9 b	20.2 a	41.9 b
Svevo	4.3 a	7.9 a	35.2 a	486.5 a	22.9 b	39.4 a
Top dressing N fertiliser						
Ammonium sulphate	4.4 a	7.8 a	36.0 a	531 b	20.6 a	40.5 a
Entec® 26	4.2 a	8.1 b	33.9 a	517 a	21.2 ab	40.6 a
Urea	4.7 b	8.2 b	36.4 a	516 a	22.8 b	41.0 a

Within treatments variety and top dressing N fertiliser, numbers followed by the same letter are not significantly different at $P \leq 0.05$.

to the number of spikes that was medium, the number of kernels per spike and the mean kernel weight that were low-medium.

Grain yield of Latinur was, on average, by 7% higher than that of Svevo (Table 3). The increase was attributed to a higher number of spikes per unit area (+14%), owing to a greater tillering rate, and mean kernel weight (+6%). Conversely, number of kernels per spike of Svevo was by 13% higher.
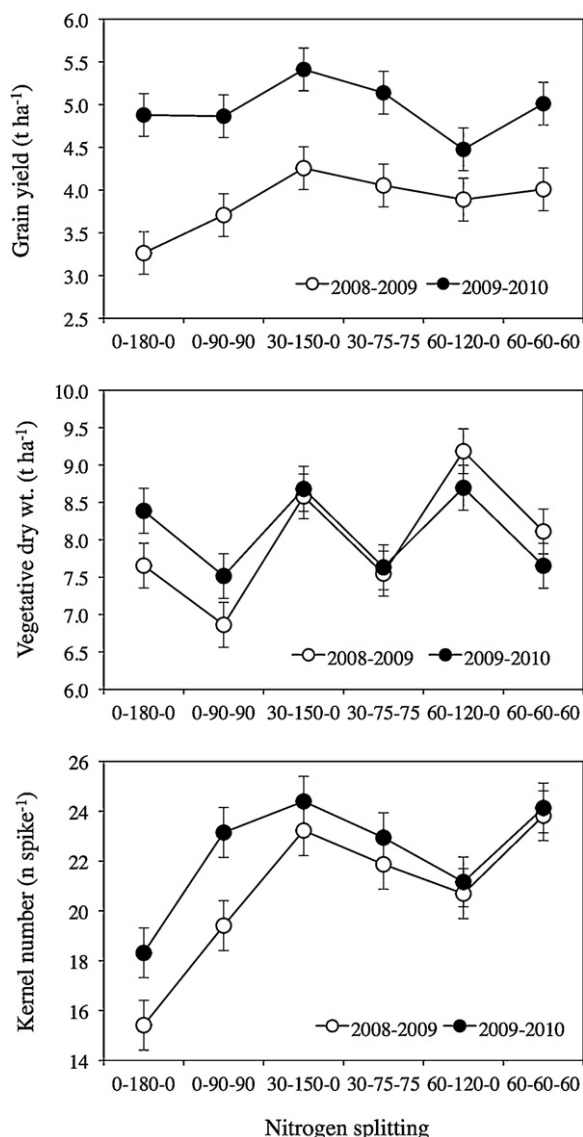

Fig. 2. Grain yield, vegetative dry weight and number of kernels per spike as affected by crop season × nitrogen splitting interaction. In these and in the following figures, error bars indicate LDS.

Grain yield was not affected by nitrogen source applied before seeding, but was mainly modified by topdressing N fertiliser. Yield increased by 0.4 t ha^{-1} with urea, compared to AS and Entec® 26 average value, owing to a greater number of kernels per spike (+9%) (Table 3).

The response of the vegetative plant part to the interaction year × splitting was different than the one of grain: dry weight was higher with 60-120-0 and 30-150-0 splittings and lower with 0-90-90 and 30-75-75 (Fig. 2). Differences between years were detected only with no N fertiliser before seeding, with higher values in 2009–2010 season. Dry weight of vegetative plant part was higher in Latinur than in Svevo and increased from AS to Entec® 26 and urea (Table 3).

Harvest Index was by 17% higher in 2009–2010 compared to 2008–2009 season and was modified by N splitting, being highest with 0-90-90 and 30-75-75 and lowest with 60-120-0 (Table 2). Differences in HI between varieties and top dressing N fertiliser were not statistically significant (Table 3).

3.3. Nitrogen uptake

Nitrogen concentration of grain and vegetative plant part were significantly affected only by the interaction year × N splitting. The highest values were found with 0-90-90 and 30-75-75 in both seasons and with 0-180-0 in 2008–2009, and the lowest with 0-180-0, 30-150-0 in both seasons and with 60-120-0 in 2009–2010 (Fig. 3). Following the patterns of dry weight and N concentration, N content of grain and vegetative plant part were affected by the interaction year × N splitting and by the mean effect of topdressing N fertiliser. Excluding the 60-120-0 split, the highest values of grain N content were recorded in 2009–2010 season at all splittings, but while in 2008–2009 differences among splittings were low (max. 18 kg N ha^{-1}), in 2009–2010 the splittings 0-90-90, 30-75-75 and 60-60-60 gave values by 28 kg N ha^{-1} higher compared to the other splittings (Fig. 3). Grain N content increased from AS to Entec® 26 (8.0%) and to urea (13.6%), even though between these last two the non-significant increase due to the urea was of 5.1 kg N ha^{-1} (Table 4). Differences for N content in vegetative plant part were also detected (Fig. 3 and Table 4). As a consequence, total N uptake by aerial plant part was by 34 kg N ha^{-1} higher with 0-90-90, 30-75-75 and 60-60-60 in 2009–2010 compared to the other

Table 4
Nitrogen content of grain and vegetative plant part. Top dressing nitrogen fertiliser mean values.

Top dressing N fertiliser	Nitrogen content (kg ha^{-1})	
	Grain	Vegetative plant part
Ammonium sulphate	92.1 a	36.9 ab
Entec® 26	99.5 ab	34.8 a
Urea	104.6 b	37.8 b

Within columns, numbers followed by the same letter are not significantly different at $P \leq 0.05$.
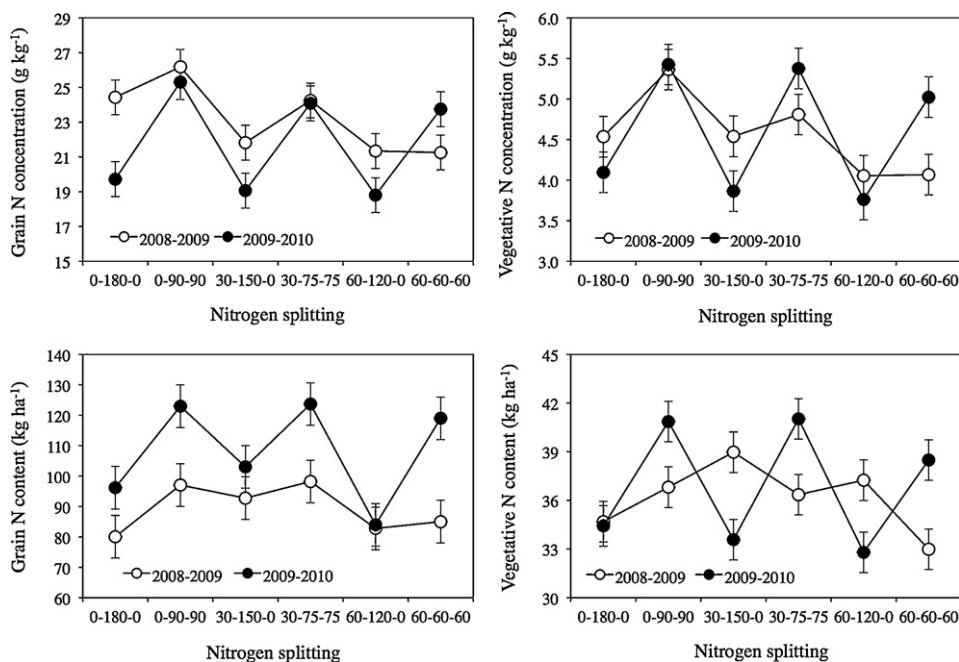
Fig. 3. Nitrogen concentration and content of grain and vegetative plant part as affected by crop season × nitrogen splitting interaction.

splittings (Fig. 3) and by 13 kg N ha^{-1} higher with urea compared to AS (Table 4). NUE was affected by the interaction year × splitting, and had the same pattern of grain yield, being the rate of fertiliser N the same for all treatments (Table 5). Similarly to grain yield, highest NUE was achieved in both year with the 30-150-0 and 30-75-75 splittings. The season influenced also NUpE and NUtE. In 2008–2009 variations due to split treatments on both indices were inconsistent, while in 2009–2010 NUpE increased appreciably with treatments involving higher N rates at GS30 (0-90-90, 30-75-75 and 60-60-60), while NUtE was higher when higher N rates were applied at GS15 (0-180-0, 30-150-0 and 60-120-0) (Table 5). The proportion of total plant N in the seeds at maturity (NHI) was lowest when the second topdressing fertilisation was not applied (Fig. 4).

3.4. Nitrogen leaching

The amount of drainage water recorded in the months from November to April was not affected by any of the tested treatments, but was higher during the 2008–2009 crop season (430.3 mm) compared to the 2009–2010 season (389.9 mm). Highest percolations occurred in December 2009 and in December and January
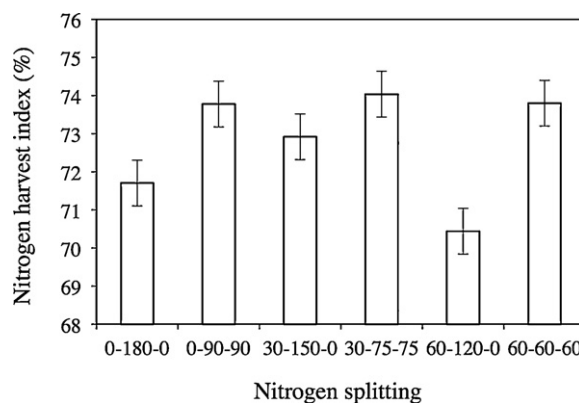

Fig. 4. Nitrogen harvest index as affected by nitrogen splitting mean effect.

2010 (Fig. 5). High rainfall determined high drainage few days following pre seeding N application in the 2008–2009 season, while in 2009–2010 drainage started about one month following N application. In November, December and January, drainage water accounted for 61% and 80% of total drainage, respectively in

Table 5

Nitrogen use efficiency (NUE), nitrogen uptake efficiency (NUpE) and nitrogen utilisation efficiency (NUtE). Season × nitrogen splitting interaction.

Season	N splitting	NUE (kg kg^{-1})	NUpE (kg kg^{-1})	NUtE (kg kg^{-1})
2008–2009	0-180-0	18.1 a	0.64 a	28.4 ab
	0-90-90	20.6 b	0.74 bc	27.7 a
	30-150-0	23.6 cd	0.73 bc	32.3 cd
	30-75-75	22.5 bc	0.75 c	30.1 abc
	60-120-0	21.6 bc	0.67 abc	32.4 cd
	60-60-60	22.3 bc	0.66 ab	34.0 d
2009–2010	0-180-0	27.1 f	0.73 bc	37.4 e
	0-90-90	27.0 ef	0.91 d	29.7 abc
	30-150-0	30.1 g	0.76 c	39.6 e
	30-75-75	28.6 fg	0.91 d	31.2 bcd
	60-120-0	24.9 de	0.65 ab	38.3 e
	60-60-60	27.8 f	0.87 d	31.8 cd

Within columns, numbers followed by the same letter are not significantly different at $P \le 0.05$.
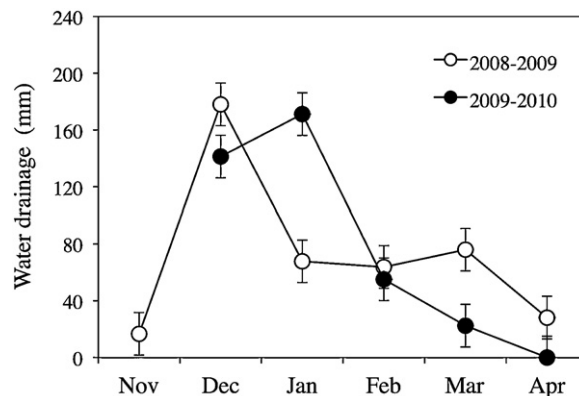

Fig. 5. Drainage water during wheat cycle as affected by crop season mean effect.
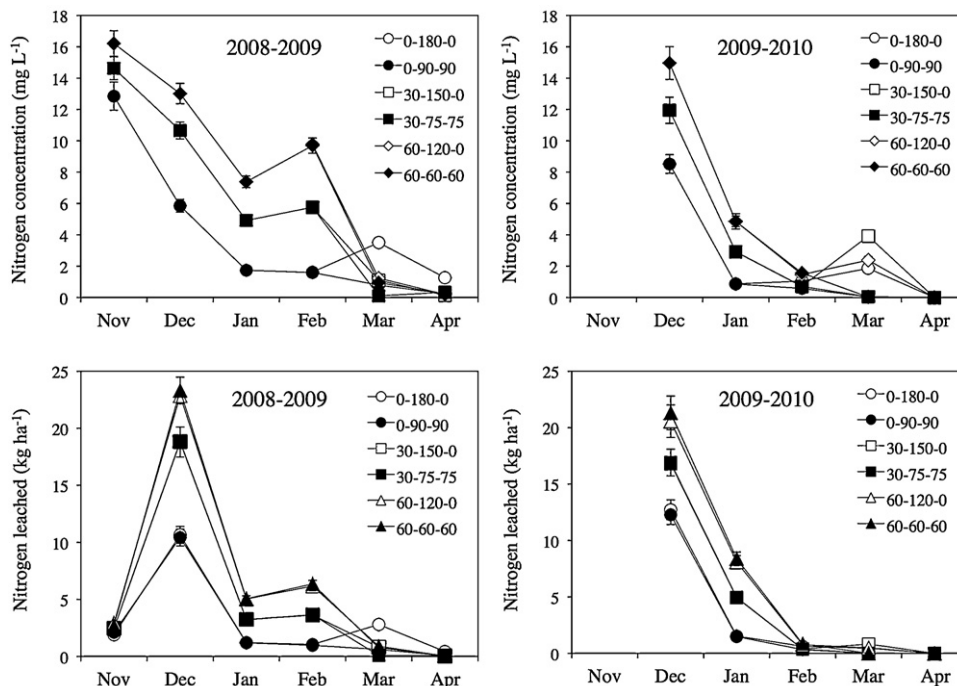
Fig. 6. Nitrogen concentration in drainage and nitrogen leached during 2008–2009 and 2009–2010 seasons as affected by nitrogen splitting mean effect.

2008–2009 and 2009–2010 seasons. The amount of drainage water was close to zero following the second top dressing fertilisation.

In both seasons, nitrogen concentration in drainage water and leached N were affected only by N splitting. Nitrogen concentration in water was higher following seeding and decreased until January in both years at all splittings (Fig. 6). In this period, N concentration increased with the increase of N rate before seeding. Thereafter, values were lower than $4\,mg\,N\,L^{-1}$ and unaffected by N splitting.

Averaged over all treatments, total amount of N lost with drainage water during wheat cycle was almost entirely accounted for N leaching in December (63% and 74% of the total leached N, respectively in the 2008–2009 and 2009–2010 seasons) (Fig. 6). In the period following first topdressing N distribution, N leached accounted for 17% and 4% of total N lost respectively for 2008–2009 and 2009–2010 seasons. The quantity of N lost in both years increased with the increase of N rate applied before seeding, while topdressing fertiliser did not affect losses (Fig. 7). When N was not applied before seeding, N lost was low and similar in the two seasons, while with the other splittings N lost was higher in 2008–2009 compared to 2009–2010 season.
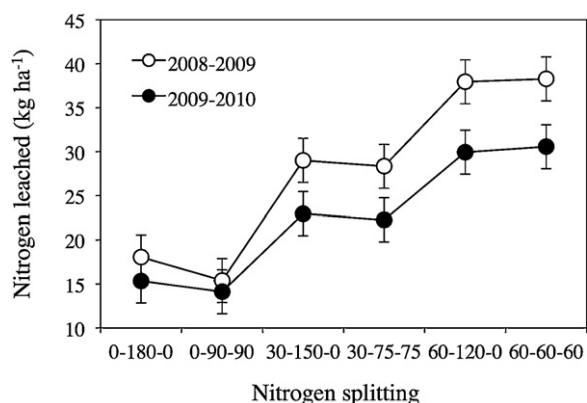

Fig. 7. Total N leached during 2008–2009 and 2009–2010 seasons as affected by nitrogen splitting mean effect.

4. Discussion

4.1. Nitrogen fertiliser at sowing

The application of SA and Entec® 46 before seeding did not modify either grain yield and yield components or N uptake and leaching, indicating that in this research the inclusion of the nitrification inhibitor did not show any advantage in comparison with ammonium fertiliser. Nitrification inhibitors have been shown to increase yield when the fertiliser is applied in conditions that favour nitrate losses by leaching, that is, in periods of high rainfall or irrigation, or in sandy or highly permeable soils (Prasad and Power, 1995; Barth et al., 2001). Conversely, in other studies the yield advantage was null or small, averaging just 5%, due to the application of N when leaching was low and when crop demand was high (Hergert and Wiese, 1980; Pasda et al., 2001; Arregui and Quemada, 2006, 2008). In this research, the application of the nitrification inhibitor was performed when leaching was high and crop demand was low, which are conditions that would favour the inclusion of nitrification inhibitors in fertiliser. Nevertheless nitrification inhibitor was not successful to control nitrate leaching during the period of high rainfall.

4.2. Nitrogen splitting

Climatic conditions during growing season influenced wheat grain yield, as in the 2008–2009 the average grain yield was by 22% lower than in 2009–2010. The high yield in 2009–2010 was due to higher number of spikes and greater spike size, probably because of good growing conditions during initial stages of crop development that favoured the development of tillers and promoted the floret fecundation, resulting in higher number of kernels per spike. Fertiliser splitting in both years had a considerable impact on grain yield and yield components. The observed differences in grain yield and yield components caused by the interactive effects of different N splittings and weather conditions may be due to processes in soil and in plant affecting N availability in critical phases of determination of yield components. Owing to the scheme of treatments (equal

total N rate, but different splittings) the effect of the different rates in different phases cannot be separated in our results, and attempts to clarify causes can only be considered as possible reasons. Nevertheless, it was demonstrated that it is possible to improve grain yield and N concentration by variations in split applications of fertiliser N: the variations due to splitting reached 1 t in grain yield while the protein grain content increased by 4%, corresponding to 7 g kg^{-1} in N concentration of grain. Comparing the effect of all splittings, the highest grain yield (and NUE) and the highest protein concentration (and NUpE) were obtained with 30-150-0 and 30-75-75 treatments, respectively. The yield advantage was related to the higher number of kernels per spike resulting from the higher number of differentiated spikelets per spike. Thus, the application of 30 kg N ha^{-1} before seeding is required to have high grain yield, as mineral N in soil deriving from mineralisation of soil organic matter and not leached probably was not sufficient to ensure high yield. The other characters were differently affected by differential N availability during crop growth, as yield components varied among split treatments in the two seasons. The application of N only topdressing (0-180-0 and 0-90-90) greatly reduced grain yield by the reduction of the number of kernels per spike. The effect was particularly heavy in the season with high rainfall before seeding, when the mineral N amount present in the soil at the time of fertiliser application was very low owing to N leaching. In the treatment 0-180-0 high N rate at GS15 stimulated tillering, but N deficiency occurred during the last part of wheat cycle (N applied at GS15 was probably immobilised into soil and not available thereafter). Nitrogen restriction during the last part of wheat cycle (0-180-0, 30-150-0, 60-120-0) lead to a reduced N accumulation into grain, and for 60-120-0 also a reduction of carbohydrate accumulation into grain. This means that grain growth was probably constrained by N shortage during grain filling. Since N leaching increased with the increase of the rate of N applied before seeding, it is likely that the lower yield with 60-120-0 compared to 0-180-0 is due to lower N availability due to N leached.

In summary, the application of N only topdressing is not a valid option since soil mineral N is not sufficient to sustain N uptake in the first three months of crop growth, when rainfall and consequently N leaching are high. Conversely, the application of the highest rate (60 kg N ha^{-1}) before seeding is not to be recommended, since it poses serious environmental concerns owing to high risk of leaching losses. Moreover, the application of N in two rates before seeding and topdressing at early crop stages is also not advisable, since dry matter and N accumulation into grain is reduced. The splitting of the N rate in three amendments instead of two with the third application at later crop stage lead to an increase of grain protein content, suggesting an improvement of grain quality parameters.

4.3. Top dressing nitrogen fertilisers

N source applied top dressing at GS15 affected grain yield and yield components, as well as dry weight of vegetative plant part and N uptake. Conversely, N leaching was not affected as the weather conditions and the crop evapotranspiration following fertiliser application did not promote NO$_3^-$ leaching. Indeed, water drainage was closely related to rainfall, which was negligible in the period following top dressing N application. The application of urea resulted in the highest grain yield, owing to a greater number of kernels per spike that compensated the lower number of spikes per unit area. In durum wheat, spike initiation occurs in the period from the development of the 4th leaf, GS14, to stem elongation, GS (Arduini et al., 2009) and the number of initiated spikelets is enhanced in presence of a good N availability in soil (Masoni et al., 2001). Our results suggest that the prompter release of N from urea compared to AS and Entec® 26 increased N available in the period of

floret initiation and differentiation. Mulvaney (1994) showed that nitrification occurred in the order urea > AS. Likewise, Eno and Blue (1957) and Martikainen (1985) reported that urea nitrifies faster than AS. This differential in nitrification rate has been attributed in part to the increase in soil pH associated with the hydrolysis of urea soon after application, in contrast to the decline in soil pH associated with the application of AS (Lindsay et al., 1962; Allred and Ohlrogge, 1964). The effect of Entec® 26 on grain yield and yield components was similar to that of AS, thus the delayed effect of the nitrification inhibitor DMPP on NH$_4^+$ nitrification did not benefit wheat growth. Entec® 26 contains 7.5% NO$_3$–N and 18.5% NH$_4$–N, while AS contains all nitrogen in NH$_4^+$ form. The probable higher persistence of NH$_4^+$ in soil in Entec® 26 than in AS plots had not any advantage for crop growth and the reduction of N–NO$_3$ due to the inhibition of nitrification in Entec® 26 compared to AS is probably compensated by the presence of nitric N. In addition, we cannot exclude that part of NH$_4^+$ may be fixed in clay minerals (Gioacchini et al., 2006), thus reducing N availability to plants.

5. Conclusions

Our results evidenced no clear advantage of the distribution to durum wheat of Entec® 46 before seeding, and Entec® 26 topdressing, neither in terms of increased grain quantity and quality, nor in terms of higher N utilisation efficiency and decreased N leaching. Since N fertiliser application represents an important cost in crop production and fertilisers containing the nitrification inhibitor DMPP have higher prices than SA or urea, their use is not advisable in Central Italy. Further researches should investigate the application to durum wheat of other delayed-release N fertilisers, focussing on the mechanisms of N release in field conditions, in order to synchronise it with plant demand.

The splitting of N fertiliser is crucial to improve fertiliser use efficiency and to increase grain yield and grain protein concentration. The application of 30 kg N ha^{-1} before seeding is recommended, since mineralisation of soil organic matter could not ensure adequate mineral N availability for initial crop development, resulting in a great reduction of kernel number per spike and consequently grain yield. Conversely, 60 kg N ha^{-1} before seeding proved to exceed crop needs, resulting in increased N leaching during initial crop stage and N deficiency at later crop development. Topdressing distribution can be recommended both entirely at 5th leaf or splitted half at 5th leaf and half at stem elongation. In our research, the first splitting, i.e. 30-150-0, gave the highest grain yield while the second, 30-75-75, gave the highest N concentration in grain. Finally, the distribution of urea at 5th leaf is advisable because, compared to ammonium sulphate and Entec® 26, it turned into higher grain yield with lower distribution costs.

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