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Proceedings

Durum wheat yield and N uptake as affected by N rate, timing and source in two Mediterranean environments. †

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Abstract: In Nitrate Vulnerable Zones (NVZ) site-specific techniques are needed to match N availability with durum wheat (*Triticum turgidum* subsp. durum Desf.) requirements.

Enhanced-efficiency fertilizers (EEF) can improve efficient N supply and reduce leaching, thus contributing to sustainable agriculture.

To study the effects of rates, sources and timings of nitrogen application, two-year field experiments were carried out at two Mediterranean NVZs of Central Italy (Pisa and Arezzo).

The trial compared: i) two N rates: one based on the crop N requirements (N_O), the other on the Action Programmes' prescriptions of the two NVZ (N_{AP}); ii) three N sources (urea, methylene urea (MU), and nitrification inhibitor (NI) 3,4-Dimethylpyrazole phosphate (DMPP); and two top-dressing timings (1st tiller visible and 1st node detectable).

Grain yield and yield components were determined, together with N uptake.

Results showed that: i) grain and biomass production were reduced with N_{AP} at both locations; ii) urea performed better than slow-release fertilizers; iii) the best application time varied depending on N source and location: at Pisa enhanced-efficiency fertilizers achieved higher yields when applied earliest, while for urea the contrary was true; at Arezzo different N fertilizers showed similar performances between the two application timings.

Different behaviors of top-dressing fertilizers at the two localities could be related to the diverse patterns of temperatures and rainfall. Therefore, optimal fertilization strategies vary according to environmental conditions.

- **Keywords:** 3,4-Dimethylpyrazole phosphate; Durum Wheat; Environmental impact; Methylene Urea; Nitrogen Management; Nitrate-Vulnerable zones; Sustainable Agriculture; Urea

1. Introduction

Nitrogen (N) is a major macronutrient often limiting plant growth, and crops yield, and quality greatly rely on extensive inputs of fertilizer nitrogen for sustainable and profitable crop production. However, N fertilization may have environmental impacts associated with nitrate leaching, eutrophication and global warming, due to the emission of nitrous oxide gases [1].

To prevent and reduce water pollution by nitrates from agricultural sources, the European Unit (EU) introduced the Nitrate Directive (ND) (91/676/EEC), a set of actions, defined at regional level, obliging Member States to designate areas vulnerable to nitrate pollution (Nitrate Vulnerable Zones - NVZ). In NVZ farmers are required to follow a range of measures, such as, among others, controlling the timing and quantities of fertilizers applied to the land [2].

In the Mediterranean areas, because of the unique site characteristics and the agricultural peculiarities, the non-point source nitrate pollution of aquifers is regarded as one of the main agricultural impacts, and N leaching as the major determinant of low N utilization efficiency (NUE) of crops.

Durum wheat (*Triticum turgidum* L. subsp. *durum*) is the most cultivated winter crop in the Mediterranean basin where is typically sown in late autumn or early winter and harvested in late spring-early summer. Mediterranean soils are typically poor in organic matter and total nitrogen content; therefore, the crop requires intensive use of N fertilizers in order to achieve sufficient yield and good grain quality [3]. In these areas, due to high rainfall and modest crop evapotranspiration rates in autumn, low N utilization efficiencies of N fertilizers have been reported, thus increasing the risks of N leaching losses [4].

Accordingly, in Mediterranean nitrate-vulnerable zones, fertilizer application matching N supply with crop demand is even more imperative, as an effective mean of achieving efficient use of N. N fertilization should be fine-tuned, concurrently aiming at enhancing both yield and its quality [5], by means of combining: i) rate, ii) splitting, iii) timing and iv) source of N application.

Excessive N application rates may not be compulsory to improve yields, as crop N use efficiency in durum wheat has been demonstrated to be low also because the N fertilizer rate often exceeds crop needs [6]. Also, for this reason, in NVZ the application of inorganic N fertilizer is limited by crop type at lower than optimal N rates, defined by yield demand and correlated to environmental conditions.

Moreover, split applications of N fertilizers have been demonstrated to improve N utilization efficiency, but the crop response is conditioned by climate and agronomic practices, like as quantity, splitting and timing of fertilizer applications together with type of fertilizer used [7, 8].

In Central Italy, the recommended timing for the first top-dressing N application to durum wheat is between late tillering and the onset of stem elongation [4]; anyway, due to climate change more frequently heavy and frequent rainfall can produce excessive soil moisture, and fertilization should be postponed. Delaying N fertilizer application may drive adverse effects on crop yield, hampering some yield determinants during the early growth stages, like the production of leaf area and the number of grains per unit area.

Enhanced-efficiency fertilizers (EEF) could be a useful tool to better synchronize N release from fertilizer with N uptake by crop, offering the potential for enhanced N use efficiency (NUE) and reduced losses to the environment [9].

Among these, slow-release fertilizers (SRF) are long-chain molecules with little solubility like formaldehyde, isobutylene diurea, or methylene urea (MU); the latter is a condensation product of urea and formaldehyde consisting of polymers with various chain lengths which allows a slow-release of N [10].

Other EEF are stabilized nitrogen fertilizers that contain nitrification inhibitors (NI) slowing the rate at which urea is hydrolyzed in the soil [10]. One of the most used nitrification inhibitors is 3,4-dimethylpyrazole phosphate (DMPP) which delays the conversion of ammonium (NH_4^+) to nitrate (NO_3^-), blocking ammonia monooxygenase, the enzyme catalyzing the first and rate limiting step of nitrification.

Anyway, field studies have shown that the efficiency of these fertilizers can significantly vary depending on the environmental conditions, because soil water content and temperature are responsible for variation in nitrification inhibitors efficiency [11, 12].

NUE may be considered as the efficiency of nitrogen recovery from applied fertilizer, or from the N available to the crop [13], otherwise like a productivity index, expressed as the yield produced per unit of available N [14, 15]. Whichever definition is used for NUE determination, it relates production as a function of inputs; thus, given constant inputs, any yield increase will be reflected in greater NUE. Accordingly, N uptake is definitely a second level trait influencing N efficiency [16]. Moreover, NHI and N content are fairly important nitrogen indexes for evaluation of slow-release

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fertilizers in crops, like durum wheat, for which N absorption (and therefore protein content) needs to be calculated [17].

Since durum wheat productivity, as well as N fertilizer use, can strongly differ among locations, based on the variability of pedoclimatic factors, effects of different N managements should be site-specifically evaluated in each NVZ, to optimize N fertilization.

We hypothesized that application of a slow-release fertilizer (MU) or a fertilizer with nitrification inhibitor (NI) could allow to foredate the first top-dressing N fertilization at tillering of durum wheat therefore preventing yield drawbacks and losses of unused N.

Overall, we aimed to evaluate i) the effects of top-dressing N application of three N fertilizers to durum wheat at two different growth stages and applied at two N rates on grain yield and N uptake and ii) to determine whether the different environments of two NVZs potentially influenced the stage at which the first N application could be applied.

2. Materials and methods

The research was carried out in open fields from November 2010 to June 2011 (2010 hereafter) and from November 2011 to June 2012 (2011 hereafter) at two experimental stations located in two different NVZs of Tuscany, Central Italy: i) the Research Centre of the Department of Agriculture, Food and Environment of the University of Pisa, (43°40 N, 10°19 E) (Pisa henceforward); and ii) the Research Centre for Agricultural Technologies at Cesa, Arezzo (Arezzo hereafter) (43°18 N, 11°48 E) (Arezzo). The climate of both sites is hot Mediterranean.

At Pisa, long-term mean annual maximum and minimum daily air temperature are 20.2°C and 9.5°C, and mean rainfall is 971 mm year^{-1}, with 515 mm received during the period of durum wheat cultivation (November-July). Soil main characteristics at Pisa were: 44.6% sand (2 mm >Ø>0.05 mm), 41.1% silt (0.05 m >Ø>0.002 mm), 14.3 % clay (Ø<0.02 mm); 8.1 pH; 2.0% organic matter (Walkley and Black method); 1.1 g kg^{-1} total nitrogen (Kjeldahl method); 9.9 mg kg^{-1} available P (Olsen method); 145.3 mg kg^{-1} available K ($BaCl_2$ + TEA method).

At Arezzo, annual maximum and minimum daily air temperature are 19.8 °C and 8.7 °C, respectively, and total annual rainfall is 755 mm, with 499 mm received during wheat growing cycle. Soil physical-chemical properties were: 17.7% sand (2 mm > Ø > 0.05 mm); 49.8% silt (0.05 m >Ø > 0.002 mm); 32.5% clay; 7.7 pH; 1.3% organic matter (Walkley and Black method); 2.7 g kg^{-1} total nitrogen (Kjeldahl method); 25.0 mg kg^{-1} available P (Olsen method); 155 mg kg^{-1} available K ($BaCl_2$ + TEA method).

In both years, daily weather data were obtained from meteorological stations located at the experimental fields. Throughout the experiment, phenological phases were recorded using the BBCH scale for cereals [18] to determine N application periods and harvesting times (Table 1).

The crop was grown following a standard technique for central Italy except from N fertilization. Soil was ploughed at 40 cm depth in September; final seedbed preparation was carried out just prior to sowing by harrowing twice, with a disc harrow and with a rotating harrow.

Sowing of variety Latinur of durum wheat was performed at both locations by means of a plot drill at the rate of 400 seeds m^{-2} on 25 and 28 November 2010 and 2011, within the optimum sowing time for wheat production in Central Italy (Table 1).

Phosphorus and potassium were applied before seeding as triple mineral phosphate and potassium sulphate at 100 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_2O.

Weed control was performed at the stage of 4th-5th leaf un-folded by distributing commercial herbicides.

The trial compared: i) three N sources for the first top-dressing application (urea, methylene urea (MU), and urea with the nitrification inhibitor (NI) DMPP); ii) two stages for the first top-dressing N application (1st tiller visible - BBCH21 and 1st node detectable - BBCH31); iii) two N rates: one based on the crop N requirements (Optimal - N_O), the other based on the Action Programmes' prescriptions of the two NVZ (Action Program - N_{AP}).

For each year and location, a randomized complete block design was used, with treatments in a split-split-plot arrangement with three replicates. N sources for the first top-dressing application were the main plots, times for the first top-dressing application were allocated as sub-plots, and N rates as sub-sub-plots. At both locations, each year a total of 36 treatments were compared with three replications (3 N fertilizers x 2 N application times x 2 N rates x 3 replications).

The optimal rates (N_O) were calculated following the balance method to achieve target yields of 5 and 6 Mg ha^{-1} at Pisa and Arezzo respectively, both with 13.5% of protein and they were 160 and 190 kg N ha^{-1} at the two locations [3, 4].

Correspondingly, the N rates based on the Action Programmes' prescriptions (N_{AP}) were 100 and 112 kg N ha^{-1} at the two NVZs. Total N rate (N_o and N_{AP}) was split into three applications: 30 kg N ha^{-1} at sowing and the remaining split into two equal top-dressing applications: the first at tillering (BBCH21) or at the 1st node detectable (BBCH31), and the second at the 2nd node detectable (BBCH32).

Fertilizers applied were: i) ammonium sulphate at sowing; ii) urea, methylene urea (MU), and urea with nitrification inhibitor (NI) 3,4-dimetihyl pyrazole phosphate – DMPP at the first top-dressing application (BBCH21 or BBCH31); iii) urea at the second top-dressing application (BBCH32).

At physiological maturity (BCCH99) plants from four adjacent rows of 1 m length were manually cut at the ground level and partitioned into culms, leaves and spikes. Spikes were counted and subsequently separated into kernels and chaff.

Mean kernel weight (MKW) and the number of kernels per unit area were also determined, and harvest index (HI) calculated as the ratio grain yield to total above-ground biomass.

The dry weight (DW) of all plant parts was measured by oven-drying at 65°C to a constant weight. All plant parts were analyzed for N concentration using the micro-Kjeldahl standard method. Total Nitrogen uptake was obtained by multiplying N concentrations of different plant parts by DW.

Nitrogen Harvest Index (NHI) was obtained as the ratio of N content in grains to the above ground N content.

Data were initially checked to verify the normality and homogeneity of variance assumptions, then ANOVA over the two years was carried out for each location. The main effects of year (Y), type of N fertilizer (S) at the first topdressing application, time of the first topdressing application (T), N rate (R), and their interactions were tested for dry weight of plant parts and relative N concentration and content. Significantly different means were separated at the 0.05 probability level by the least-significant difference test [19].

Table 1. Durum wheat growth stages in the two growing seasons (2010 and 2011) at the two locations.

Stage	BCCH	Pisa First season	Pisa Second season	Arezzo First season	Arezzo Second season
Sowing	00	25 Nov 2009	28 Nov 2010	25 Nov 2009	28 Nov 2010
Tillering[1]	21	10 Feb 2010	19 Feb 2011	6 Mar 2010	10 Mar 2011
1st node[1]	31	25 Mar 2010	28 Mar 2011	8 Apr 2010	8 Apr 2011
2nd node[2]	32	9 Apr 2010	13 Apr 2011	22 Apr 2010	26 Apr 2011
Full Flowering	65	2 May 2010	6 May 2011	12 May 2010	16 May 2011
Maturity	99	12 July 2010	16 July 2011	21 July 2010	23 July 2011

[1] 1st top-dressing application; [2] 2nd top-dressing application.

3. Results

3.1.Weather conditons

At both locations, temperatures were similar in the two years and close to the long-term average (Figure 1). Maximum and minimum temperatures were higher at Pisa than at Arezzo in winter; in spring maximum temperatures were similar between the two locations while minimum temperatures were lower at Arezzo with the maximun difference being in the last decade of March in both years.

At Pisa, rainfall during the crop cycle was similar in the two years (about 475 mm) and slightly lower than the long-term average (515 mm). At Arezzo, rainfall differed between years and was 413

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mm and 351 mm in 2010 and 2011, correspondingly similar to, and 30% lower, than the long-term average (499 mm).

3.2 Grain yield

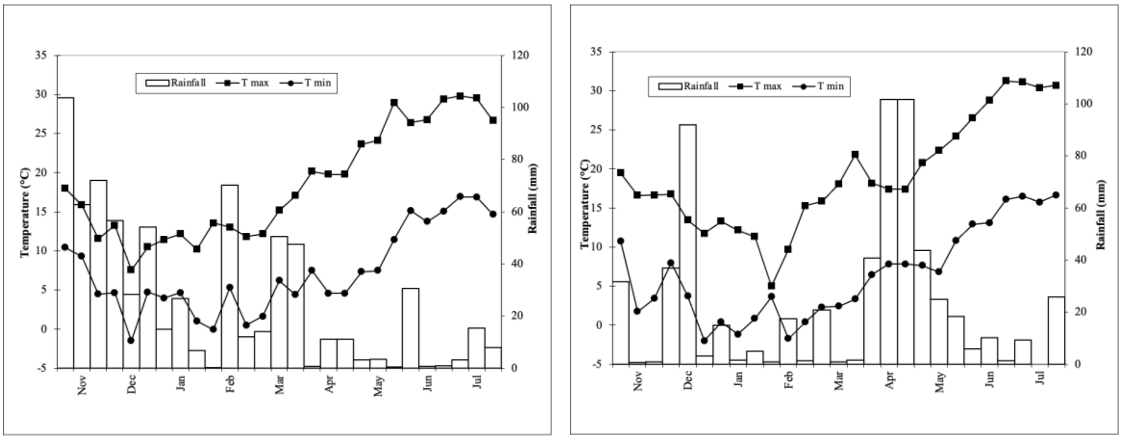
The analysis of variance revealed significant differences among treatments at the two localities, for some of the analyzed characters, as summarized in Table 2. Anyway, to enhance concision and intelligibility, only some of the main results are here reported and discussed, referring to further presentations for residual topics.

3.2.1 Year effect

Significant differences between years at both locations for some of the measured parameters were shown, but none of the interactions with year was significant (Table 2).

Durum wheat was similarly affected by year at the two NVZ of Pisa and Arezzo. The crop produced 30% more dry biomass in vegetative aboveground parts (VAP) and 39% higher grain yields in the first season at Pisa, and 24% and 27% at Arezzo respectively (Table 3). What is more, for both sites, the yield rise was due to an increase in the spikes produced per unit area (2-fold at Pisa and about +14% at Arezzo) and to a slight increase (less than 10%) in the MKW, which together overbalanced the reduced number of kernels per spike at Pisa (-37%). At Arezzo kernels per spike were not different between the two years.

Total N uptake of the crop was similarly boosted in the first season (+18 and 9% respectively at Pisa and Arezzo), even if NHI did not differ between years at the two locations (Table 3).



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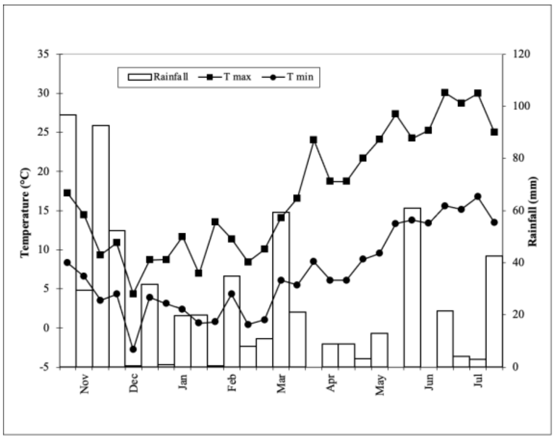

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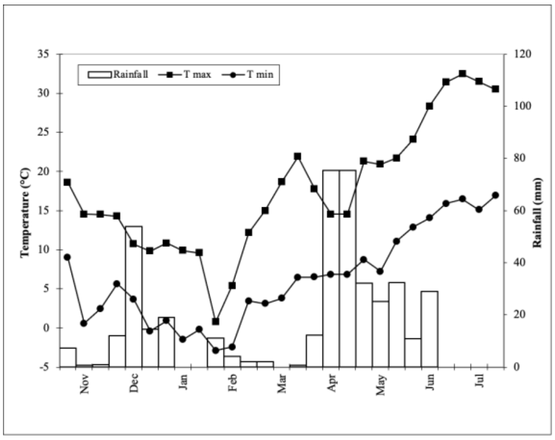

(d)

Figure 1. Maximum and minimum temperature and rainfall recorded in the two durum wheat growing seasons at the two locations: (**a**) Pisa first season (November 2010 – June 2011); (**b**) Pisa second season (November 2011 – June 2012); (**c**) Arezzo first season (November 2010 – June 2011); (**d**) Arezzo second season (November 2011 – June 2012).

Table 2. Results of ANOVA for durum wheat vegetative aboveground part (VAP), grain yield, yield components and NHI and N uptake as affected by Year (Y), N source (S), N timing (T), N rate (R) and their interactions at the two locations.

	VAP	**Grain**	**H.I.**	**Spikes**	**Kernels**	**MKW**	**NHI**	**N uptake**
	Mg ha^{-1}	Mg ha^{-1}	%	n m^{-2}	n spike^{-1}	mg	%	kg ha^{-1}
				Pisa				
Y	*	*	ns	*	*	*	ns	*
S	*	*	ns	*	*	ns	ns	ns
T	*	ns	*	ns	ns	ns	ns	ns
R	*	*	ns	ns	*	*	*	*
Y x S	ns	ns	ns	ns	ns	ns	ns	ns
Y x R	ns	ns	ns	ns	ns	ns	ns	ns
Y x T	ns	ns	ns	ns	ns	ns	ns	ns

S x R	ns	*	ns	*	ns	ns	ns	*
S x T	*	*	ns	*	ns	ns	ns	ns
R x T	ns	ns	ns	ns	ns	ns	ns	ns
Y x S x R	ns	ns	ns	ns	ns	ns	ns	ns
Y x S x T	ns	ns	ns	ns	ns	ns	ns	ns
Y x R x T	ns	ns	ns	ns	ns	ns	ns	ns
S x R x T	ns	ns	ns	*	*	*	ns	ns
Y x S x R x T	ns	ns	ns	ns	ns	ns	ns	ns
				Arezzo				
Y	*	*	ns	*	ns	*	ns	ns
S	ns	ns	ns	ns	ns	ns	ns	ns
T	ns	ns	ns	ns	ns	ns	ns	ns
R	*	*	ns	*	ns	*	ns	*
Y x S	ns	ns	ns	ns	ns	ns	ns	ns
Y x R	ns	ns	ns	ns	ns	ns	ns	ns
Y x T	ns	ns	ns	ns	ns	ns	ns	ns
S x R	ns	ns	ns	ns	ns	*	ns	*
S x T	ns	ns	ns	ns	ns	ns	ns	ns
R x T	ns	*	ns	ns	*	ns	ns	ns
Y x S x R	ns	ns	ns	ns	ns	ns	ns	ns
Y x S x T	ns	ns	ns	ns	ns	ns	ns	ns
Y x R x T	ns	ns	ns	ns	ns	ns	ns	ns
S x R x T	ns	ns	ns	ns	ns	ns	ns	ns
Y x S x R x T	ns	ns	ns	ns	ns	ns	ns	ns

Table 3. Dry weight of vegetative above ground parts (VAP), grain yield, Harvest Index (HI), yield components and Nitrogen Harvest Index (NHI) and total N uptake as affected by Year (Y), at Pisa and at Arezzo. For each location, values followed by different letters within lines are significantly different ($P < 0.05$).

Character	**u.m.**	**Pisa**				**Arezzo**			
		2010		**2011**		**2010**		**2011**	
VAP	*Mg ha^{-1}*	4.3	a	3.3	b	6.5	a	5.2	b
Grain	*Mg ha^{-1}*	3.5	a	2.5	b	5.2	a	4.1	b
HI	%	44.7	a	43.1	b	44.6	a	43.9	b
Spikes	*n m^{-2}*	405.6	a	194.3	b	461.8	a	338.0	b
Kernels	*n spike^{-1}*	24.1	b	38.1	a	29.2	b	33.8	a
MKW	*mg*	35.5	a	33.8	b	38.5	a	35.6	b
NHI	%	73.7	ns	72.1	ns	73.6	ns	72.8	ns
N uptake	*kg ha^{-1}*	83.5	a	70.8	b	124.8	a	114.2	b

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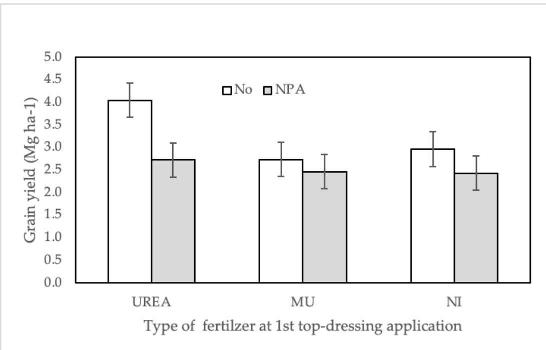
3.2.2 N source, N rate and N timing effects

The type of the fertilizer used at the first top-dressing N application (N source) drove differences in vegetative above ground part (VAP) and in grain yield at Pisa but not at Arezzo (Table 2). Moreover, at Pisa N source produced different effects depending on the rate and timing of application, as also N source x N rate, and N source x N timing interactions were significant in determining durum wheat production (Table 2). Grain yield was higher with the optimal rate only when fertilization was performed with urea, as it didn't change when MU or NI were used (Figure 2a). The same was true for the timing of the first top-dressing fertilization (Figure 2c): urea performed better when applied at 1st node detectable (BBCH31), while MU and NI fertilizers produced higher yields at the earlier stage. Both effects were due to the more spikes developed by the crop, that were maximed with urea applied at N_o rate (Figure 2b) and at BCCH31 (Figure 2d).

N source did not significantly influence any of the studied characters at Arezzo (Table 2) neither the N source x N timing interaction was significant. Conversely, at this location, grain yield was affected by N rate x N timing interaction (Table 2): the N optimal rate prompted 12% higher grain yield when the 1st top-dressing application was at BCCH21, mainly due to 21% more kernels per spike (Figure 3a and 3b).

No differences were detected between the two growth stages of N application with the Action Programme rate (N_{AP}).



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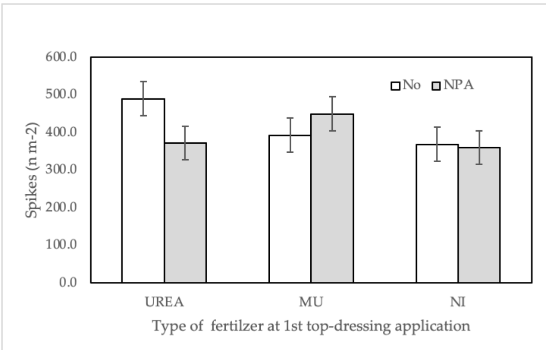

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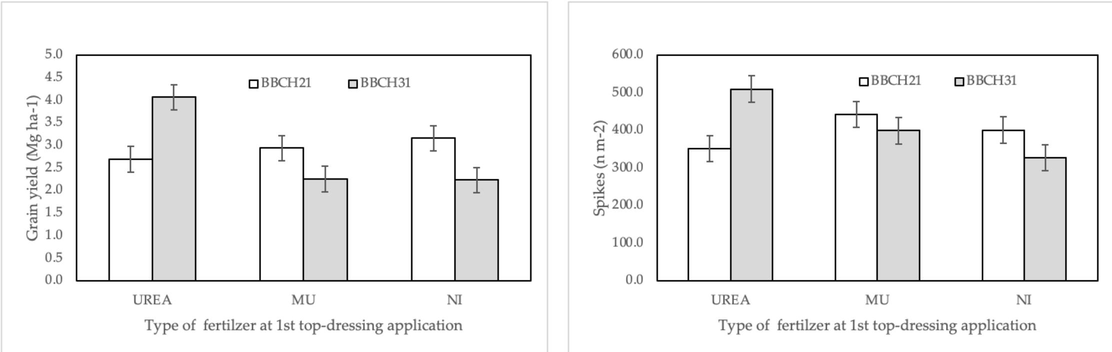
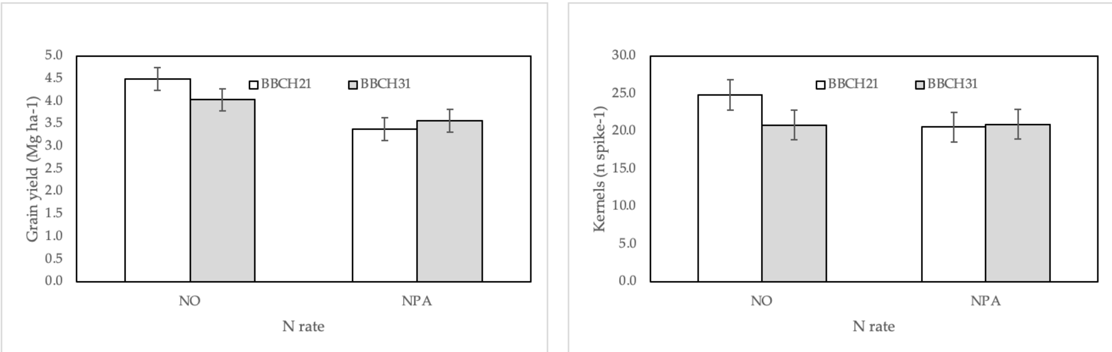


(c) (d)

Figure 2. N source x N rate interaction effect at Pisa: (**a**) Grain yield (N source x N rate interaction); (**b**) Spike number per unit area (N source x N rate interaction); (**c**) Grain yield (N source x N timing interaction); (**d**) Spike number per unit area (N source x N timing interaction). Vertical bars represent LSD (p = 0.05).



(a) (b)

Figure 3. N rate x N timing interaction effect at Arezzo: (**a**) Grain yield; (**b**) Kernel number per spike. Vertical bars represent LSD (p = 0.05).

Generally, higher N rates increased grain yield at both sites, as predictable (Table 2); and, averaged over years, stages and timings, optimal N rates (N_O) produced grain yields of 3.9 Mg ha^{-1} at Pisa and 5.7 Mg ha^{-1} at Arezzo, corresponding to rises of about 25% (+28 and +23%), compared to the rates of the Application Programmes (N_{AP}), that yielded 3.1 and 4.7 Mg ha^{-1} respectively.

Superior yield with the N_{AP} was due to more kernels produced per spike (27.0 with N_O and 21.2 with N_{AP}) that counteracted for a sligthly lower MKW (34.6 mg instead of 36.5 mg) at Pisa; while at Arezzo the lower MKW (37.4 mg versus 39.6 mg) was compensated by more spikes produced per unit area (500.2 spikes m^{-2} with N_O and 423.3 with N_{AP}).

3.3 NHI and Nitrogen uptake

At Pisa, partitioning of nitrogen between grain and straw was changed only by the amount of N given (N rate) (Table 2): the optimal N rate caused a lower NHI (69.9%) than the Action programme rate (74.2%); while at Arezzo NHI was not affected by any of the treatments.

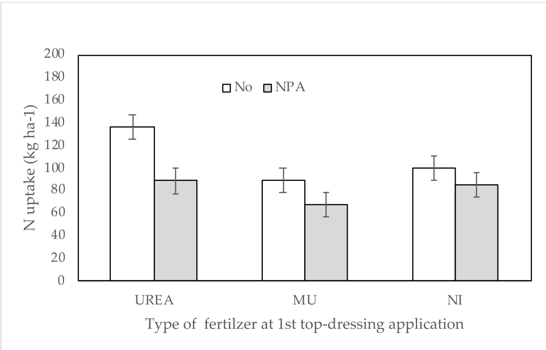
Total N uptake of the crop changed between years at Pisa, increasing by 18% in the first season (83.5 kg ha^{-1} in 2010 vs 70.8 kg ha^{-1} in 2011), as a result of the higher biomass produced. However, at Arezzo N uptake was not significantly different in the two experimental seasons (Table 2).

What is more, at both locations N source differently affected the N uptake of the crop, depending on the total N applied (N source x N rate interaction – Table 2).

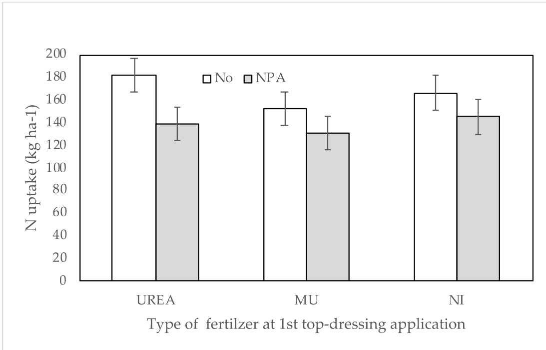
At Pisa and Arezzo, durum wheat maximized the N uptake when urea was applied at the 1st top-dressing event and at the optimal N rate (N_o); this treatment increased the N uptake of durum wheat by 54% and 31% respectively at Pisa and Arezzo compared to the reduced N rate (N_{AP}) (Figure 4a and 4b).

At Pisa, N uptake differed depending on the N rate, when methylen urea was applied. With the optimal rate, the crop showed 32% higher N uptake, mainly imputable to differences in grain N content (69.5 kg ha^{-1} with N_o vs 50.1 kg ha^{-1} with N_{Ap}); interestinlgy at this location MU at the N_{AP} rate, showed the lowest N uptake among all the treatments (Figure 4a).

Finally, the N fertilizer with NI did not show differences between the two N rates, at any site.



(a)



(b)

Figure 4. N uptake of durum wheat as affected by N source x N rate interaction at the two locations: (**a**) Pisa; (**b**) Arezzo. Vertical bars represent LSD (p = 0.05).

4. Discussion

Overall, our results confirm that durum wheat yield is influenced by seasonal variation in climatic conditions [4]. In the first season, grain yield was 38 and 28% higher at the two localities, due to the higher number of spikes produced per unit area. Likely, in 2011, the erratic rainfall and lower temperatures of December – January did not promote tillers development, resulting in less spikes produced. Additionally, excessive rainfall during seed filling lowered the mean kernel weight and the N content of grains that triggered also a minor total N uptake, according to our previous findings in durum wheat [20].

So far, the main purpose of this study was to define N management practices to better synchronize N supply with crop N uptake in durum wheat cropping systems at two Nitrate Vulnerable Zones in Central Italy.

Regarding the N source, urea endorsed 28% higher grain yields than the two slow-release fertilizers at Pisa, while at Arezzo different N sources caused very similar yields. Therefore, our

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results did not show any agronomic benefit from using methylene urea or nitrification inhibitors over conventional urea applied at the 1st top-dressing event, at any of the two locations.

High water contents in soil, as probably resulted in the present experiment due to high rainfall, may have reduced the efficiency of DMPP as suggested by literature [21]. Similar previous findings revealed that yield components and nitrogen use efficiency were not improved by NI in durum wheat [4]. Moreover, the increase in NUE for wheat at a range of 9% after the introduction of nitrification and urease inhibitors was not necessarily linked with an increase in grain yield [22].

Results obtained at Pisa highlighted that the application of urea increased the number of spikes per unit area. Likely, the use of this fertilizer at top-dressing accounted for better N availability in soil during the spike initiation period, that in durum wheat takes place from the development of the 4th leaf to the stem elongation, probably because of the short period for mineralization of the N fertilizer, from its application to the spike initiation. Whichever the mechanism involved, the main feature of the two EEF fertilizers is that N takes longer to become available to the plants. Under the present experimental conditions, this may have lowered the N available in soil for durum wheat, at the critical stage when the crop N demand increases sharply (just prior the onset of the most rapid phase of crop growth, that is stem elongation). Shortage of N during this period reduced subsequent shoot development and tillering, leaded to reduced spike formation and thus, depressed final grain yield.

This effect was also evidenced from the interaction between the type of fertilizer and the application timing: the two slow-release fertilizers performed better when applied earlier (at 1st tiller BBCH21), conversely urea endorsed higher results with the later distribution (at 1st node detectable BCCH31).

In line with increased yields, N uptake was stimulated by the application of urea, indicating a positive effect on N use efficiency, and supporting our above-mentioned hypothesis. Similar results for durum wheat have also been reported by [23].

Differences among fertilizers were not evidenced at Arezzo, probably because the lower temperatures recorded at Arezzo may have constrained urea hydrolysis on one side, and DMPP action on the other [21, 24]. This can be confirmed by the similar performances of the different N fertilizers between the two growth stages of 1st top-dressing application.

Also, the differences in soil characteristics at the two sites can be responsible of variable yields responses to N sources. Higher clay content and lower pH at Arezzo may have reduced the effect of the NI and improved MU microbial decomposition as suggested by [24], then N available in soils from the EEF resulted similar to that from urea and the crop obtained similar yields and N uptakes.

Finally, our results generally pointed out that the two enhanced-efficiency fertilizers showed comparable results and, despite their different mode of action [25], as a matter of fact, both of them did not permit a N release as fast as urea. Whereas common urea fertilizer likely underwent to rapid hydrolysis [26], for the MU fertilizer, its conversion to plant-available N is a multistep and longer process, involving dissolution and decomposition [24]; and for the other fertilizer, the addition of the NI slowing down the hydrolysis of urea, retarded the nitrification of ammonium [27].

With respect of N rate, the lower N rates defined by the Action Programmes of the two Nitrate Vulnerable zones had a detrimental effect in both growing seasons and at both sites. Generally, durum wheat with N_{AP} showed reduced biomass and grain yield and their N content compared to N_o. However, since reductions in these characters were proportional, mean HI and NHI were similar.

In the present research NHI ranged between 68 to 74% and was influenced only by the N rate at one location (Pisa), confirming that it is principally determined by genotype, in durum wheat [28, 29, 30].

Anyway, at Pisa, grain yield was improved with the optimal rate only when the top-dressing fertilization was performed with urea. Given that when MU or NI were used, the two N rates were comparable, reducing the amount on N given is possible without yield constrains, if N source is properly chosen.

At Arezzo, differences in grain yield due to N rate were higher when the 1st top-dressing fertilization was done earlier, probably because the less rainfall at this site could have lowered N-leaching. As a consequence, more N remained in the soil for a longer time, and the crop was able to absorb additional N, as confirmed by the greater N uptake.

Conclusively, the optimization of N fertilization is a central issue in the global challenge for meeting increased food demand and protecting environment in the frame of sustainable agriculture. This could be achieved with the 4R approach - right source, right amount, right time and right placement [31].

In the present research, we aimed to evidence the effects of N source, rate and timing on durum wheat yields and N uptake, and concluded that the use of methylene urea and nitrification inhibitors is a potentially attractive approach to improve fertilizer performance, but without a notable increase in yield and N use efficiency compared to conventional urea, it may not be economically feasible in durum wheat, unless positive environmental factors like decreased leaching of N are confirmed.

Anyway, optimal N fertilization strategies depended on site-specific environmental conditions.

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Abbreviations: EEF, enhanced-efficiency fertilizers; MU, methylene urea; N, nitrogen; NI, nitrification inhibitor; DMPP, 3,4-dimethylpyrazole phosphate; NUE, Nitrogen Use Efficiency; NHI, Nitrogen Harvest Index; NVZ, Nitrate Vulnerable Zone; SRF, slow-release fertilizers.

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