Effect of a nitrification inhibitor (DMPP) on nitrate leaching and maize yield during two growing seasons

J. A. Díez-López^{1*}, P. Hernaiz-Algarra¹, M. Arauzo-Sánchez¹ and I. Carrasco-Martín²

¹ Centro de Ciencias Medioambientales CSIC-CCMA. C/ Serrano, 115. 28006 Madrid. Spain ² COMPO Agricultura. C/ Joan d'Austria, 39-47. 08005 Barcelona. Spain

Abstract

The aim of this experiment was to evaluate the effects of adding DMPP (3,4-dimethylpyrazole phosphate) to urea on nitrate leaching and maize (*Zea mays* L.) yield over two growing seasons. Two nitrogen (N) levels (optimum and excessive) were applied to an irrigated maize crop under Mediterranean conditions. There were five treatments: optimal N (as urea) and the same dose plus DMPP; optimal N (as urea plus 40 kg N ha⁻¹) and the same N dose plus DMPP; and a control with no added N fertilizer. The maize was irrigated with an overhead mobile-line sprinkler system. EnviroSCAN probes were used to determine drainage and evapotranspiration and ceramic cups to obtain soil solution samples at a soil depth of 1.4 m. The use of DMPP with urea reduced nitrate leaching. No phytotoxic effects were observed due to the DMPP. There were no differences in grain yield between treatments with and without DMPP at the same rate of N. When DMPP was applied, sodium was displaced from the soil exchange complex due to increased NH₄⁺ concentration, which also increased the electrical conductivity of soil in the drainage zone.

Additional key words: aquifer pollution, control nitrate leaching, irrigation, urea.

Resumen

Efecto de un inhibidor de la nitrificación (DMPP) sobre la lixiviación de nitrato y la producción de maíz, durante dos periodos de cultivo

El objetivo de este experimento fue evaluar los efectos de la adición de DMPP (3,4-dimetilpirazol fosfato) a la urea sobre la lixiviación de nitrato y la producción de maíz durante dos periodos de cultivo. Para su realización se aplicaron dos niveles de nitrógeno (óptimo y en exceso) a un maíz irrigado, bajo condiciones mediterráneas. Se aplicaron cinco tratamientos: una dosis óptima de nitrógeno en forma de urea, la misma dosis de urea con DMPP, una dosis óptima de urea más 40 kg N ha⁻¹ y la misma dosis con DMPP; y un testigo sin fertilización nitrogenada. Para el riego del maíz, se empleó un sistema mediante Pívot. Se emplearon sondas de EnviroSCAN para determinar el drenaje y la evapotranspiración; y cápsulas cerámicas de vacío para obtener muestras de la solución del suelo a una profundidad de 1,4 m. El uso de DMPP con la urea redujo la lixiviación de nitrato. No se observó ningún efecto fitotóxico en el cultivo debido al inhibidor, pero tampoco se obtuvieron diferencias en la producción de grano entre tratamientos con y sin DMPP a la misma dosis de nitrógeno. Cuando se aplicó DMPP, el sodio fue desplazado del complejo de cambio debido al aumento de la concentración de NH₄⁺, el cual también originó un incremento en la conductividad eléctrica en la zona de drenaje.

Palabras clave adicionales: contaminación de acuíferos, control de nitrato lixiviado, riego, urea.

Introduction¹

Nitrification inhibitors are compounds that delay ammonium oxidation by reducing the activity of *Nitro-somonas* bacteria in the soil. These bacteria transform

ammonium into nitrite, which in turn is oxidized to nitrate by *Nitrobacter* bacteria (Trenkel, 1997). Due to partial ammonium nutrition, plants can use other pathways to build up their biomass. Ammonium can be more efficiently metabolized than NO₃ because it

^{*} Corresponding author: jadiez@ccma.csic.es Received: 09-07-07; Accepted: 10-03-08.

¹ Abbreviations used: ASN (ammonium sulfate nitrate), DCD (dicyandiamide), DM (dry matter), DMPP (3,4-dimethylpyrazole phosphate), EC (electrical conductivity), ETo (evapotranspiration), EUF (electro ultrafiltration), HI (harvest index), SAR (sodium adsorption ratio).

does not need to be reduced when incorporated into amino-acids or other organic compounds. However, NH₄⁺ is toxic to plants at certain concentrations (Magalhaes and Wilcox, 1984) and this toxicity is related to soil pH. Olsen (1986) cited several studies where addition of ammonium to a nitrate system increased maize (*Zea mays* L.) yield. Teiker and Hobbs (1992) reported that with coarse-textured soils of slightly alkaline pH, an enhanced NH₄⁺ regime may be advantageous for maize growth. The object of using nitrification inhibitors is to prolong the presence of N in the soil as ammonium which is fixed in the clay-humic complex, and thus increase the efficiency of applied N.

The compound 3,4-dimethylpyrazole phosphate (DMPP) is a nitrification inhibitor developed by BASF (Limburgerhof Research Centre, Germany), which inhibits only the first stage of nitrification. It can be added to both conventional fertilizers and slurries: it is highly specific in its action, and only a small amount (0.8% of applied N) is needed to inhibit nitrification for several weeks. Zerulla et al. (2001) showed the physical and chemical properties of DMPP. The duration of its action depends on temperature and humidity conditions (Pasda et al., 2001). It can remain effective in upper soil layers even after heavy rain (Fettweis et al., 2001). DMPP has passed all toxicological and ecotoxicological tests that it has been submitted to (Roll, 1999) and has proved to be highly plant compatible (Zerulla et al., 2001). Further, it offers other benefits in crop production from both the economic (labour savings) and environmental (less nitrate leaching and lower N gas emissions) points of view. Homogenous growth, high quality fruits and reduced nitrate contents in vegetables, like spinach (Spinacea oleracea L.) and lettuce (Lactuca sativa L.), (Zerulla et al., 2001) are other advantages obtained from using fertilizers with DMPP. However, Barth et al. (2001) concluded that the benefits of DMPP are limited to coarse-textured soils.

Nitrate leaching is normally intensified by excessive rain or irrigation as well as by use of N levels which are surplus to crop requirements (Díez *et al.*, 1997). The amount of available soil N (Sánchez *et al.*, 1998) and crop N requirement must be known to establish the optimum application rate.

Some authors have shown that use of DMPP, as a nitrification inhibitor, enables more efficient N fertilizer utilization in citrus-cultivated soils (Serna *et al.*, 2000). This was mainly achieved through regulation of N supply, allowing more continuous nitrate release to the soil, and through reduced N loss due to nitrate leaching.

The aim of this work was to evaluate the effect of adding DMPP to urea at a concentration of 0.8% (w/w) on nitrate leaching and N uptake and irrigated maize yield over two years. Two N levels, optimum and excessive, were applied to an irrigated maize crop under Mediterranean conditions to analyze soil and groundwater N contents and N leaching and to show that application of different levels of DMPP did not depress maize yield.

Material and Methods

Experimental site

The experimental site was at the La Poveda Field Station in Arganda del Rey (Madrid) (40° 19'N, 3°19'W), in the middle of the Jarama river basin. The soil, a *Typic* Xerofluvent (Soil Survey Staff, 1993), was a sandyloam that became progressively sandier with depth and had a gravel layer at a depth of 1.5-2.2 m. Some of the physicochemical characteristics of the top 0-50 cm are shown in Table 1. Soil samples were analyzed for pH, organic matter (Walkley and Black, 1934) and carbonate (ISO 10693, 1995). Nitrogen, phosphorus (P), K and calcium (Ca) levels were estimated using electroultrafiltration (EUF) (Nemeth, 1979). Total N was determined from EUF extracts (EUF-N) of soil samples by digestion with UV radiation and subsequent oxidation with potassium persulphate in an alkaline medium (Díez, 1988). The P level was also colourimetrically determined using ammonium molybdate as a reagent (AOAC, 1990). Potassium and Ca levels were determined by flame emission photometry, texture by ISO 11277 (1998) and bulk density by ISO 11272 (1998) (Table 1).

Table 1. Physicochemical properties of the soil before sowing

Descriptor	Mean ± SD	
pH (H ₂ O)	8.1 ± 0.1	
Organic matter (g kg ⁻¹)	14.0 ± 0.2	
EUF-P 20°C (mg P 100g ⁻¹)	1.48 ± 0.2	
EUF-K 20°C (mg K 100g ⁻¹)	12.25 ± 2.1	
EUF-N ^a (mg N 100g ⁻¹)	8.37 ± 1.5	
CaCO ₃ (g kg ⁻¹)	34.0 ± 0.8	
EUF-Ca 20°C (mg Ca 100g-1)	39.0 ± 2.4	
Sand (%)	38.7 ± 5.6	
Silt (%)	47.5 ± 9.0	
Clay (%)	13.8 ± 5.2	
Bulk density (Mg m ⁻³)	1.47	

^a Both extracted fractions.

The depth of the water table fluctuated from 4-4.5 m below the soil surface depending on rainfall and river discharge. Maximum temperatures at the Field Station during growth of the maize crops was between 11.5 and 38.4°C in 2004, and 14.5 and 40.6°C in 2005. Minimum temperatures were 1.5 to 12.4°C in 2004 and 2.7 to 19.1°C in 2005. Average rainfall in the area is 460 mm yr⁻¹.

Experimental design, field instrumentation and crops

Maize (cv. Tector) cycle 700 (Novartis) was grown at the experimental site in 2004 and 2005. Fifteen 100 m² experimental plots were selected and five treatments with three randomized replicates were applied in the first year. In the second year the plots received the same treatments as in the first year with the N dose reviewed. The treatments applied were: an optimal rate of urea (U_1) and the same N dose plus DMPP $(U_1$ -DMPP); an optimal N rate of urea plus 40 kg N ha⁻¹ (U₂) and the same N dose plus DMPP (U2-DMPP); and a control given no N fertilizer (C). Based on soil analysis, by EUF and criteria established by Sánchez et al. (1998), the optimal N rate (Table 2) for the maize crops was 160 and 220 kg N ha⁻¹ in 2004 and 2005, respectively. The optimal N rate was low (160 kg N ha⁻¹) especially in 2004, when it was calculated by considering the soil residual N and mineralized N during crop growth. In 2005, the rate was 220 kg N ha⁻¹ because the amount of soil residual N was lower. These rates were lower than those traditionally used by farmers in this area of

Table 2. Soil available N in the two seasons and rate of N applied in each treatment based on optimal N requierements (kg N ha⁻¹)

2004	2005
150	80
160	220
160	220
200	260
200	260
	150 160 160 200

^a Available N calculated by soil analysis at 0-20 cm using the EUF method before sowing and having previously calibrated the soil according to its N balance (Sánchez *et al.*, 1998). This includes mineral N + potential mineralizable N. ^b Optimal N rates were calculated through the expression: (N absorption by crop – available N)/N efficiency (Sánchez *et al.*, 1998). ^c DMPP: 3,4 dimethylpyrazole phosphate.

300 kg N ha^{-1} for maize. Optimal N rates (U_1) were calculated by:

Available soil N was calculated by soil analysis (EUF) before sowing (Sánchez *et al.*, 1998). Nitrogen efficiency was the % of N fertilizer used by the crop, which in this case had previously been estimated at 70% (Díez *et al.*, 2000). Treatments were applied once, after sowing, by topdressing. They were applied on 11 June 2004 and on 4 June 2005. Application rates are given in Table 2. A net was installed during early maize growth to protect the crop from birds.

The maize was sown at the end of April in both years. Rows were 75 cm apart and the plant density was 90,000 plants ha⁻¹. During seedbed preparation, superphosphate and potassium sulphate were applied at 21.8 kg P ha⁻¹ and 99.5 kg K ha¹. The maize was grown using traditional farm practices in the area, except for N fertilization. It was harvested at grain maturity in October. Experimental plots were hand weeded in May.

Monitoring soil water content and drainage

Water used during the experiment was from an irrigation channel fed by the Jarama River. This water was sampled 18 times during the experiment. The average quality components of the irrigation water were: NO $_3$, 5.1 ± 0.5 mg N L $^{-1}$; Na, 90 ± 16 mg L $^{-1}$; total solids, 650 ± 50 mg L $^{-1}$; electrical conductivity (EC), 1.0 ± 0.1 dS m $^{-1}$; Na adsorption ratio (SAR), 1.55; and pH, 7.6 ± 0.2. The groundwater contained: NO $_3$ -, 2.7 ± 1.0 mg N L $^{-1}$; and Na $^+$, 70 ± 25 mg L $^{-1}$.

The maize was irrigated with an overhead mobile-line sprinkler system. Irrigation started on 19 June 2004 and on 9 June 2005 and was continued until the end of August. The maize was watered every 7-10 days following the schedule traditionally used by most growers in the area. The amount of water applied through irrigation was that recommended by the local advisory services, and was based on a potential evapotranspiration (ETo) of 500-600 mm for the dry season (ITAP, 2004). The final water rate was calculated to obtain moderate water losses. Water was applied ten times (mean 51 mm) during the 2004 dry season and eleven (mean 69 mm) times in 2005 due to higher temperatures.

A year before the experiment began, a system for monitoring soil water content in real time using semipermanent multisensor capacitance probes (EnviroSCAN, Sentek Pty Ltd, South Australia) (Buss, 1993) was installed. Drainage was calculated as:

$$D = R + I - ET \pm \Delta S$$
 [2]

where: D is drainage (mm), R is the rainfall (mm), I is irrigation (mm), ET is evapotranspiration (mm) and ΔS is the change in the soil water reserve (mm) from 0 to 50 cm depth. Four of the fifteen plots, corresponding to different treatments, were monitored using EnviroSCAN probes (50 mm interior diameter) at a depth of 150 cm. The sensors, situated inside the probes, were at 10, 40, 70, 120 and 150 cm depth. The frequency signal (FS) from the device was converted into a percentage of volumetric moisture (θv). The equipment was calibrated for the experimental soil, using the calibration equation of Paltineanu and Starr (1997). In both years the equipment was programmed to take hourly readings throughout crop growth. Data was recorded by a data logger. Drainage was calculated from descent water reserve curves obtained from the EnviroSCAN data corresponding to sensors situated near the drainage zone (150 cm depth) and from the water balance between layers. Four water flow patterns were identified and six water balance equation partitioning patterns were reported (see Román et al., 1996).

Sampling

Treatments were randomized across the experimental area and analyzed individually. Soil samples were taken from depths of 0 to 0.20 m in 2004 and at 2, 32, 56 and 93 days after N application. In 2005, samples were taken 2, 13, 29, 43 and 62 days after N application. Samples were air dried, ground, extracted using 1M KCl at a ratio of 1:5. NH₄⁺ and NO₃⁻ and the contents of the drainage water were determined directly from the extracted aliquots.

Samples of the soil solution were collected in ceramic cups at a depth of 1.4 m and extracted 23 times during the course of the experiment. A vacuum of –80 kPa was applied to the tubes and maintained for a period of 7 to 10 days. After this period, water samples were extracted using air pressure. Nitrate, Na⁺ concentration, and EC were subsequently determined. For the nitrate leaching study, two ceramic cups were used to

obtain soil solution samples from each plot at a depth of 1.4 m (Díez et al., 2001). It was considered that any water reaching this level, near the gravel layer, was leached into the groundwater (at an average depth of 4 m) because of the high hydraulic conductivity (Smith et al., 1991). During drainage periods, NO₃-leaching was calculated on a weekly basis by multiplying the weekly drainage by the corresponding NO₃-concentration at 1.4 m for each sampling event (Díez et al., 1997). Estimation of drainage volume has been discussed by Román et al. (1999). The NO₃ concentration was determined with a Technicon AAII Autoanalyzer (Technicon Hispania) using the N naphthylethylenediamine method (AOAC, 1990), Na⁺ concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer 403, Perkin-Elmer Hispania), and electrical conductivity (EC) with a Crison 525 conductivity meter.

Maize plants were harvested from the central 5 m of the rows in each plot. Aboveground biomass was determined. Ten harvested maize plants were randomly selected and divided into stalk, leaves, bracts, cob and grain. They were weighed, oven-dried for 24 h at 60°C, then kept for a further 2 h at 80°C before reweighing to determine their dry matter (DM) content. The harvest index (HI) was calculated as grain weight over aboveground biomass (percentage). Grain yield kg ha⁻¹ was calculated and plant N content determined (Díez *et al.*, 2001).

Statistical analysis

Statistical analysis was performed using STATGRA-PHICS Plus 5.1 software (Manugistics, 2000). Analysis of variance, used multivariate models to study differences between datasets, agronomic data (plant DM at harvest, grain yield and plant N content) and soil solution data (EC, NO_3 - and Na+ concentrations). This datasets passed the normality test. Differences between seasons and among treatments were analyzed and compared using the Duncan test. Significance was set at P < 0.05.

Results

Soil nitrogen

Two days after N application, soil NH₄⁺ concentrations were at their highest in all treatments, and

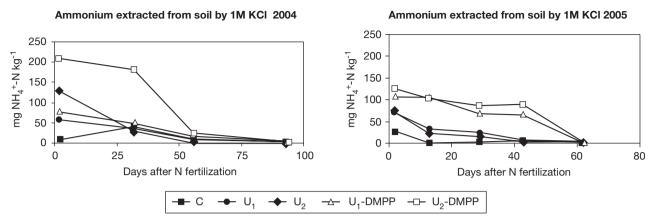


Figure 1. The NH_4^+ extracted from soil by 1 M KCl in 2004 and 2005, at different times after N fertilization (means of three replicates). Treatments: C, U_1 -DMPP, U_1 , U_2 -DMPP and U_2 are unfertilized control, optimum rate of urea with DMPP, optimum rate of urea, optimum rate of urea plus 40 kg N ha⁻¹ with DMPP and optimum rate of urea plus 40 kg N ha⁻¹, respectively.

especially in plots treated with DMPP. Soil U_2 -DMPP values were 206 and 124 mg NH_4^+ -N kg^{-1} in 2004 and 2005, respectively (Fig. 1). The NH_4^+ concentration was significantly lower in plots that received urea alone (U_1 and U_2) than in plots that had received DMPP-treatments (soil U_2 values of 129 and 77 mg NH_4^+ -N kg^{-1} in 2004 and 2005, respectively). In all treatments the NH_4^+ concentrations fell after the initial increase and reached the same level as the unfertilized control plots after 60 d. The changes in soil NH_4^+ content were similar in both years, although the level of NH_4^+ was lower in 2005.

In contrast, nitrate levels (Fig. 2) increased significantly in the first 15-30 d after fertilization in treatments without DMPP, while treatments including DMPP had

NO₃⁻ concentrations similar to those of the unfertilized control.

Nitrate leaching

Under conditions of the experiment, 70-80% of the water applied during the two years was used by the crop, although drainage was higher in 2004 than in 2005. The amounts of irrigation water applied to the maize crops in 2004 and 2005 were 518 and 642 mm, respectively, based on soil water reserves (Table 3) and drainage losses were 201 and 91 mm, respectively, representing an average drainage loss equivalent to 25% of total irrigation water applied. In 2004, the drainage loss was

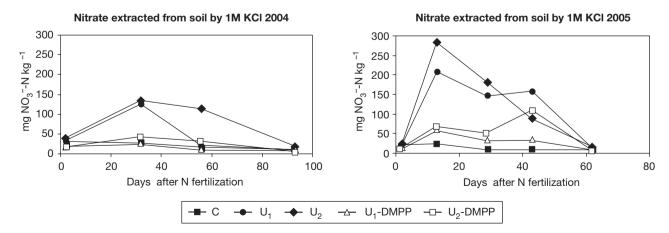


Figure 2. The NO_3^- extracted from soil by 1 M KCl in 2004 and 2005, at different times after N fertilization (means of three replicates). C, U_1 -DMPP, U_1 , U_2 -DMPP and U_2 are: unfertilized control, optimum rate of urea with DMPP, optimum rate of urea, optimum rate of urea plus 40 kg N ha⁻¹ with DMPP and optimum rate of urea plus 40 kg N ha⁻¹, respectively.

Table 3. Rainfall, irrigation, evapotranspiration (ET) and drainage during crop growth (mm)

Year	Rainfall	Irrigation	ETa	Drainage ^a
2004	168	518	618	201
2005	63	642	743	91

^a Calculated from descent curves of water reserves EnviroSCAN data corresponding to sensors situated near the ET zone (at depths of 20 and 50 cm). Drainage (at a depth of 140 cm) was calculated from the water balance between different soil layers.

higher due to tests to establish the effect of DMPP on NO₃- leached under conditions of heavy drainage. The differences between inputs and losses, of water in the system, are due to soil water reserves prior to the crop period.

Table 4 shows that, regardless of N source, soil solution nitrate concentration increased significantly with increased N application. However, DMPP treatments were always associated with a lower NO_3 -concentration at similar rates of N application. Drainage water from fertilizer plots contained high NO_3 -levels (between 80 and 240 mg NO_3 -L-1) and very low NH_4 + levels (between 0 and 0.9 mg NH_4 + L-1).

Soil solution nitrate concentration data at a depth of 1.4 m (Table 4) were used to study the possibility of groundwater pollution. Cumulative NO₃- discharge at 1.4 m mainly depended on irrigation water applied and the fertilizer treatment used (Fig. 3). As observed by Díez *et al.* (2000), total leaching mainly depended on drainage and, to a lesser extent, on variation in NO₃- concentration at the percolation depth.

N uptake and grain yield

The effect of fertilizer treatments with and without DMPP on maize dry matter, N uptake and maize yield in two seasons is shown in Table 5. There were no significant differences among treatments in 2004 with respect to plant N uptake. In 2005, there was a significant increase in N uptake between the U_2 -DMPP and U_1 treatments. These results could not prove that unleached N accumulated in the soil due to the nitrification inhibitor being taken up by the plants, although an increasing trend was observed in the DMPP treatments.

Discussion

The inhibitory effect of DMPP on nitrification resulted in NH₄⁺ accumulation in two months. This is analogous with the results of Serna *et al.* (2000) who found NH₄⁺ from ammonium sulphate nitrate that N was present in the soil for up to 60 d when using DMPP. However, these results should be analyzed considering the observed temperatures during the experiment (maximum temperature 38.4°C in 2004 and 40.6°C in 2005). Temperature was reported by Frye *et al.* (1989) as a limiting factor for other nitrification inhibitors (DCD: dicyandiamide). Perhaps the same effect could be associated with DMPP application (Zerulla *et al.*, 2001). Consequently it can be concluded that temperatures during the experiment did not depress the effect of DMPP, due especially to the irrigation water.

Table 4. ANOVA between seasons (2004 and 2005) and treatments. Values of mean nitrate concentration^a, electrical conductivity (EC) and Na concentration in soil solution at a soil depth of 1.4 m

Parameters ANOVA		C ^b	U ₁ DMPP	\mathbf{U}_{1}	U ₂ DMPP	$\mathbf{U_2}$	
NO ₃ - conc. (mg NO ₃ -N L-1)	Season Treatments	F=2.29; NS ^c F=87.92; P<0.05	38.8a	67.0b	143.3c	153.0c	188.3d
EC (dS m ⁻¹)	Season Treatments	$F = 3.25$; NS^c F = 63.31; $P < 0.05$	2.39a	4.62c	3.42b	6.94d	4.58c
Na conc. (mg Na L ⁻¹)	Season	F = 6.20; P < 0.05 2004 2005 453a 520b					
	Treatments	F = 63.97; P < 0.05	235a	563c	359b	844d	432b

^a Data based on 6 replicate ceramic cup extractions at a soil depth of 1.4 m (23 samplings over the two seasons). Means followed by different letters in each row indicate significant differences among treatments. ^b C, U₁-DMPP, U₁,U₂-DMPP and U₂ are unfertilized control, optimal urea rate, optimal urea rate + DMPP, optimal N rate of urea + 40 kg N ha⁻¹, optimal N rate of urea + 40 kg N ha⁻¹ + DMPP, respectively. ^c Not significant.

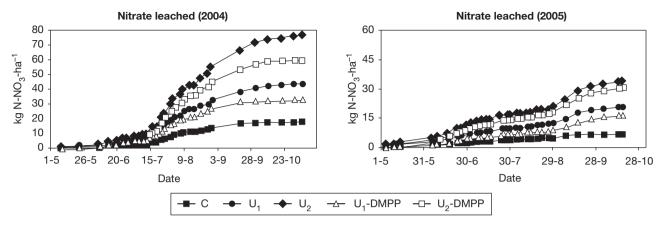


Figure 3. Nitrate leaching during maize crop growth in 2004 and 2005 for the various N treatments (C, U_1 -DMPP, U_1 , U_2 -DMPP are unfertilized control, optimum rate of urea with DMPP, optimum rate of urea, optimum rate of urea plus 40 kg N ha⁻¹ with DMPP and optimum rate of urea plus 40 kg N ha⁻¹, respectively). Values are means of four drainage replicates and six nitrate concentration replicates. Date = day-month.

Over the course of the experiment there were no appreciable changes in NO₃⁻ concentration between the drainage (after rainfall and irrigation) and non-drainage periods. However, great spatial variability in NO₃⁻ concentration was observed both within a given plot and among plots. This confirmed the findings of Kengni *et al.* (1994), Bruckler *et al.* (1997) and Díez *et al.* (2001).

Significant differences (P<0.05) among treatments were recorded with respect to NO₃⁻ leaching in 2004, with losses of 18 (a), 32 (b), 44 (bc), 60 (c) and 76 (d) kg NO₃-N ha⁻¹ for C, U₁-DMPP, U₁, U₂-DMPP and U₂,

respectively (different letters in brackets indicate significant differences among treatments, Duncan test. There were also significant differences (P < 0.05) among treatments in 2005, with losses of 6 (a), 16 (ab), 21 (c), 32 (cd) and 34 (d) kg NO₃-N ha⁻¹ for C, U₁-DMPP, U₁, U₂-DMPP and U₂, respectively. Consequently, more attention must be paid to drainage than to the N fertilizer dose. Taking into account that in both years the N rate applied was calculated from available soil N, in 2004 more NO₃⁻ was released than in 2005 due to more drainage, despite the higher N application in 2005. Nitrogen losses due to leaching as a % of N applied

Table 5. Effect of fertilizer nitrogen treatments with and without DMPP on maize dry matter yield, N uptake and maize grain yield in 2004 and 2005

Year	Treatment ^a	Dry matter (Mg ha ⁻¹)	Plant N uptake (kg ha ⁻¹)	Grain yield (Mg ha ⁻¹)
2004	С	21.6a ^b	152a	11.6a
	U_1	29.0b	253b	16.5b
	U_1 -DMPP	30.0b	259b	17.5b
	U_2	30.1b	290b	18.8b
	U ₂ -DMPP	31.9b	296b	18.9b
	P	< 0.05	< 0.05	< 0.05
2005	С	16.1a	114a	8.1 a
	U_1	23.5b	225b	14.3b
	U_1 -DMPP	23.6b	234bc	14.6b
	U_2	24.4b	243bc	14.8b
	U2-DMPP	25.0b	257c	14.7b
	P	< 0.05	< 0.05	< 0.05

^a C, U_1 -DMPP, U_1 , U_2 -DMPP and U_2 are unfertilized control, optimal rate of urea with DMPP, optimal rate of urea, optimal N rate of urea plus 40 kg N ha⁻¹ with DMPP and optimal N rate of urea plus 40 kg N ha⁻¹, respectively. ^b Different letters in a column for each year indicate significant treatment differences (P < 0.05, Duncan test).

in fertilizer (discounting N leached from control plots) were 9%, 17%, 26% and 37% in 2004 and 5%, 7%, 10%, and 11% in 2005 for U₁-DMPP, U₁, U₂-DMPP and U₂, respectively. These results show that with accurate control of irrigation and the use of nitrification inhibitors, it is possible to reduce water loss by drainage and consequently to reduce the quantity of nitrate leached. Under high temperature conditions DMPP can reduce nitrate pollution, despite the fact that it limits the application of nitrification inhibitor (DCD) to maize (Frye et al., 1989). In this experiment, NO₃- losses due to leaching were much more important than those produced by denitrification (N₂O) that were reported by Vallejo et al. (2004) in a parallel experiment on the same site and at the same time.

As expected, treatments with DMPP (U_1 -DMPP and U_2 -DMPP) maintained higher soil N levels than the urea (U_1 and U_2) treatments, especially in soil volumes occupied by roots (Serna *et al.*, 2000). These authors also reduced N losses through NO_3^- leaching, using DMPP. When N loss is high, due to high drainage, as in 2004, a greater response to DMPP can be expected. Figure 3 shows that in 2004 where there were greater losses due to NO_3^- leaching, there were appreciable differences among treatments involving DMPP (smaller losses) and those with urea alone. However, the differences were smaller in 2005, when there was less drainage.

The EC and sodium results indicated that when applying DMPP, Na may be displaced from the soil exchange complex due to increased NH₄⁺. It can then be leached and results in increased EC in the drainage zone.

The EC in the drainage zone of DMPP treated plots increased significantly during the experiment (3.42 and 4.62 dSm⁻¹ in the U_1 and U_1 -DMPP treatments, respectively; 4.58 and 6.93 dSm⁻¹ in the U_2 and U_2 -DMPP, respectively) (Table 4). At the same time, increased Na concentration was observed at 1.4 m depth in treatments involving DMPP (359 and 563 mg Na l⁻¹ in the U_1 and U_1 -DMPP treatments, respectively and 432 and 844 mg Na L⁻¹ in U_2 and U_2 -DMPP treatments, respectively).

The ANOVA shows that there were no significant differences between seasons with respect nitrate concentration and EC (Table 4). However, there were significant differences in Na concentration between 2004 and 2005. In 2005 the Na concentration was higher, due to the cumulative effect of Na displaced from the soil exchange complex in 2004.

In both seasons there were significant differences (P < 0.05) among the control and the fertilized treatments

with respect to dry matter and grain yield (Table 5). Although there were no significant differences among fertilized treatments, plots to which DMPP was applied, generally had higher dry matter and grain yields. This is coherent with that of Pasda *et al.* (2001) «in some crops, the same yield level was obtained in the treatment with DMPP as in the treatment without DMPP with one fewer application of N, or with a reduced N application rate». In contrast, the same authors reported increased crop yield associated with the use of DMPP for various agricultural and horticultural crops, including maize. This generally occurred at high N application rates. The results obtained here show that DMPP did not increase decrease maize grain production when surplus DMPP was applied (U₂+DMPP).

In conclusion, application of DMPP increased soil NH₄⁺ content. The inhibitory effect of DMPP with respect to nitrification resulted in NH₄⁺ accumulation over a period of 60 d. Consequently, NO₃-levels significantly increased in the first 15-30 d after fertilization in treatments without DMPP, while those with DMPP produced NO₃ concentrations similar to the unfertilized control. The use of N fertilizers with DMPP reduced the amount of nitrate leached. There were greater NO₃ losses in 2004 than in 2005 due to greater drainage, in spite of the N rate applied being higher in 2005. No significant increases in maize grain production or dry matter were observed following application of DMPP. There was no yield depression due to high rates of DMPP. When N fertilizer was applied once, as a topdressing it gave good maize dry matter and grain yields. When DMPP was applied, sodium was displaced from the soil exchange complex, resulting in an increase in the EC in the drainage zone. Ammonium fertilization using DMPP could therefore alleviate soil salinity by the release of Na to deeper layers of the soil profile.

Acknowledgments

We acknowledge the assistance of A. de Diago and his collaboration with laboratory work. Part of this work was supported by the Spanish Ministry of Science and Technology Grant CICYT AGL2001-2214-C06-02.

References

AOAC, 1990. Official method of analysis. Association of Official Analytical Chemists. 13th ed. Arlington. VA.

- BARTH G., VON TUCHER S., SCHMIDHALTER U., 2001. Influence of soil parameters on the effect of 3,4-dimethyl-pyrazole-phosphate as a nitrification inhibitor. Biol Fertil Soils 34, 94-102.
- BRUCKLER L., DE COCKBORN A.M., RENAULT P., CLAUDOT B., 1997. Spatial and temporal variability of nitrate in irrigated salad crops. Irrig Sci 17, 53-61.
- BUSS P., 1993. The use of capacitance based measurements of real time soil water profile dynamics for irrigation scheduling. Proc Natl Conf Irrig Assoc Australia and the Ntl. Committee of Irrig and Drainage, Launceston, Tasmania. 17-19 May. Homebush, NSW.
- DÍEZ J.A., 1988. Revisión del método de determinación automatizado de nitrógeno UV oxidable en extractos de suelo. Anal Edafol Agrobiol 47, 1029-1039. [In Spanish].
- DÍEZ J.A., ROMÁN R., CABALLERO R., CABALLERO A., 1997. Nitrate leaching from soils under a maize-wheat-maize sequence, two irrigation schedules and three types of fertilisers. Agr Ecosyst Environ 65, 189-199.
- DÍEZ J.A., CABALLERO R., ROMÁN R., TARQUIS, A., CARTAGENA M.C., VALLEJO A., 2000. Integrated fertiliser and irrigation management to reduce nitrate leaching in Central Spain. J Environ Qual 29, 1539-1547.
- DÍEZ J.A., DE LA TORRE A.I., CARTAGENA M.C., CARBALLO M., VALLEJO A., MUÑOZ, M.J., 2001. Evaluation of the application of pig slurry to an experimental crop using agronomic and ecotoxicological approaches. J Environ Qual 30, 2165-2172.
- FETTWEIS U., MITTELSTAEDT W., SCHIMANSKY C., FÜHR F., 2001. Lysimeters experiments on the translocation of carbono-14-labelled nitrification inhibitor 3,4 dimethylpyrazole phosphate (DMPP) in a gleyic cambisol. Biol Fertil Soils 34, 126-130.
- FRYE W.W., GRAETZ B.A., LOCASCIO S.J., REEVES D.W., TUCHTON J.J., 1989. Dicyandiamide as a nitrification inhibitor in crop production in Southeastern USA. Commun Soil Sci Plant Anal 29, 1969-1999.
- ISO 10693, 1995. Soil quality. Determination of carbonate content. Volumetric method. International Organization for Standardization, Switzerland.
- ISO 11272, 1998. Soil quality. Determination of dry bulk density. International Organization for Standardization, Switzerland.
- ISO 11277, 1998. Soil quality. Determination of particle size distribution in mineral soil material. Method by sieving and sedimentation. International Organization for Standardization, Switzerland.
- ITAP, 2004. Boletín Instituto Técnico Agronómico Provincial. Diputación de Albacete, Servicio de Asesoramiento de Riegos, Albacete, Spain. [In Spanish].
- KENGNI L., VACHAUD G., THONY J.L., LATY R., GARINO B., CASABIANCA H., JAME P., VISCOGLIOSI R., 1994. Field measurements of water and nitrogen losses under irrigated corn. J Hydrol 162, 23-46.

- MAGALHAES J.R., WILCOX G.E., 1984. Ammonium toxicity development in tomato plants relative to nitrogen form and light intensity. J Plant Nutr 7, 1477-1496.
- MANUGISTICS, 2000. Statgraphic Plus Version 5.1 Manugistics, Rockville, USA.
- NEMETH K., 1979. The availability of nutrients in the soil as determined by electroultrafiltration (EUF). Adv Agron 31, 155-187.
- OLSEN S.R., 1986. The role of the organic matter and ammonium in producing high corn yields. In: The role of the organic matter in modern agriculture (Chen Y., Avnimelech Y., eds). Develop Plant Soils Sci, Martinus Nijhoff, The Hague. pp. 29-54.
- PALTINEANU C., STARR J.L., 1997. Real-time soil water dynamics using multisensor capacitance probes: laboratory calibration. Soil Sci Soc Am J 61, 1576-1585.
- PASDA G., HÄHNDEL R., ZERULLA W., 2001. Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. Biol Fertil Soils 34, 85-97.
- ROLL R., 1999. Zur Toxikologie von DMPP. In: Düngen mit einer neuen Technologie Innovation in der Düngung ENTEC. Proc Scientific Colloquium (BASF AG, ed), 17-18 May. BASF Agricultural Centre, Limburgerhof, Scientific. pp. 39-44. [In German].
- ROMÁN R., CABALLERO R., BUSTOS A., DÍEZ J.A., CARTAGENA M.C., VALLEJO A., CABALLERO A., 1996. Water and solutes movement under conventional corn in Central Spain. I. Water balance. Soil Sci Soc Am J 60, 1530-1536.
- ROMÁN R., CABALLERO R., BUSTOS A., 1999. Field water drainage under traditional and improved irrigation schedules for corn in Central Spain. Soil Sci Soc Amer J 63, 1811-1817.
- SÁNCHEZ L., DÍEZ J.A., VALLEJO A., CARTAGENA M.C., POLO A., 1998. Estimate of mineralized organic nitrogen in soils using nitrogen balances and determining available nitrogen by the electroultrafiltration technique. J Agric Food Chem 46, 2036-2043.
- SERNA M.D., BAÑULS J., QUIÑONES A., PRIMO-MILLO E., LEGAZ F., 2000. Evaluation of 3,4-dimethylpyrazole phosphate as a nitrification inhibitor in a *Citrus*cultivated soil. Biol Fertil Soils 32, 41-46.
- SMITH K.A., MULLINS C.E. (ed), 1991. Soil analysis. Physical methods. Marcel Dekker, New York.
- SOIL SURVEY DIVISION STAFF, 1993. Soil survey manual. Soil Conservation Service, US Department Agriculture, Handbook 18.
- TEIKER R.H., HOBBS D.C., 1992. Growth and root morphology of corn as influenced by nitrogen form. Agron J 84, 697-700.
- TRENKEL M.E., 1997. Controlled-release and stabilized fertilizers in agriculture. In: Improving fertilizer use efficiency. Int Fert Ind Assoc (IFA), Eusserthal, Germany. pp. 1-123.
- VALLEJO A., DÍEZ J.A., LÓPEZ-VALDIVIA L.M., CARTAGENA M.C., TARQUIS A., HERNAIZ P., 2004. Denitrification from an irrigated soil fertilized with pig

- slurry under Mediterranean conditions. Biol Fertil Soils 40, 93-100.
- WALKLEY A., BLACK A.I., 1934. An examination of the Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid titration method. Soil Sci 37, 29-38.
- ZERULLA W., BARTH T., DRESSEL J., ERHARDT K., HORCHLER VON LOCQUENGHIEN K., PASDA G., RÄDLE M., WISSEMEIER A.H., 2001. 3,4-dimethylpyrazole phosphate (DMPP) a new nitrification inhibitor for agriculture and horticulture. Biol Fertil Soils 34, 70-84.