

Comparison of nitrification inhibitors to restrict nitrate leaching in a maize crop irrigated under mediterranean conditions

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Abstract

The aim of this paper was to compare dicyandiamide (DCD) and 3,4 dimethylpyrazole phosphate (DMPP) as inhibitors of ammonium oxidation and nitrate leaching after applying fertilizer to a maize (*Zea mays* L.) crop grown under Mediterranean conditions. The effects of nitrification inhibitors were also compared to those of N fertilization without inhibitors and with split N application. In plots fertilized with ammonium sulphate nitrate (ASN), either DCD or DMPP lengthened ammonium presence in soil and produced lower soil NO₃⁻ concentrations (30% lower than in plots with no inhibitor). The use of DCD or DMPP achieved significant reductions in nitrate leaching. DCD showed excellent properties for controlling nitrate leaching, taking into account the fact that grain yield and N accumulated by plant were similar for the ASN-DCD and ASN treatments applied at the same N doses. The split N treatment did not offer any advantages in terms of leached nitrate, either with the use of single ammonium sulphate nitrate (ASN) or with single application of nitrification inhibitors. The nitrification inhibitors did not increase the yield but did not reduce it either. The drainage rate was the most important component of nitrate leaching. The low drainage values of the first year resulted in a sharp decline of nitrate leaching. However, the experiment of the second year, showed clear differences in nitrate leaching between treatments due to the greater drainage.

Additional key words: dicyandiamide, dimethylpyrazolephosphate, N fertilization, split N application.

Resumen

Comparación de dos inhibidores de la nitrificación para reducir la lixiviación de nitrato en un cultivo de maíz irrigado bajo condiciones mediterráneas

El objetivo de este trabajo fue comparar el efecto de la diciandiamida (DCD) y el 3,4 dimetilpirazolfosfato (DMPP) como inhibidores de la nitrificación, en la fertilización nitrogenada de un cultivo de maíz (*Zea mays* L.) bajo condiciones mediterráneas. Se compararon los efectos de los inhibidores frente a la fertilización sin inhibidores y con aplicaciones divididas de nitrógeno. En parcelas fertilizadas con nitrato sulfato amónico (ASN), tanto DCD como DMPP, prolongaron la presencia de amonio en el suelo, originando un descenso en las concentraciones de NO₃⁻ (30% menos que en parcelas fertilizadas sin inhibidor) y en consecuencia en la lixiviación de nitrato. El DCD mostró excelentes propiedades para controlar la lixiviación de nitrato, ya que la producción de grano y el N acumulado por la planta fueron similares para el tratamiento ASN-DCD y ASN, ambos aplicados a la misma dosis de N. El tratamiento de N en dosis divididas no ofreció ninguna ventaja sobre el nitrato lixiviado ni frente al uso de ASN solo, ni frente a la aplicación de ASN con alguno de los inhibidores de la nitrificación. En el experimento se observa que los inhibidores de la nitrificación no aumentan la producción pero tampoco la reducen. La dosis de drenaje fue el factor más importante en la lixiviación de nitrato. Los bajos valores de drenaje del primer año experimental originaron bajos valores de nitrato lixiviado. Sin embargo, en el segundo año, los valores de drenaje mas elevados dieron mayores niveles de lixiviación de nitrato y permitieron observar claras diferencias entre tratamientos.

Palabras clave adicionales: diciandiamida, dimetilpirazolfosfato, fertilización dividida de N, fertilización nitrogenada.

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Abbreviations used: ASN (ammonium sulphate nitrate), C (control), CAP (Common Agricultural Policy), CRD (completely randomized design), DCD (dicyandiamide), DM (dry matter), DMPP (3,4 dimethylpyrazole phosphate), EC (electrical conductivity), ET_c (crop evapotranspiration), ET_o (reference crop evapotranspiration), EUF (electroultrafiltration), FDR (frequency domain reflectometry), FS (frequency signal), HI (harvest index), SAR (Na adsorption ratio), SP (split application of ASN).

Introduction

It is necessary to control nitrate leaching resulting from agricultural practices in order to protect or improve water quality, but effective management is difficult because of the complex interactions between soil, water and nitrogen (Zerulla *et al.*, 2001). The declaration of vulnerable zones to nitrate pollution (CD 91/676/EEC; OJ, 1991) and the inclusion of protecting water from nitrate pollution as conditions for obtaining Common Agricultural Policy (CAP) support have made farmers more aware of the need to control this problem. Maize crops have a great demand for water and nitrogen, particularly under Mediterranean conditions, and irrigation is therefore a common practice.

Nitrification inhibitors have been used at low concentrations (Ashworth *et al.*, 1982; Amberger, 1991) with the aim of reducing nitrate leaching. In such cases, the strategy was to hold ammonium in the soil thereby retarding its oxidation to nitrate. The use of nitrification inhibitors may offer an alternative to splitting N applications (Boswell *et al.*, 1976) in order to improve the efficiency of applied N.

Dicyandiamide, $\text{H}_2\text{NC}(\text{NH})\text{NHCN}$ (DCD), a dimmer of cyan-amide or cyan-guanidine, is an effective nitrification inhibitor (Ashworth *et al.*, 1982). DCD contains about 67% N and is non-volatile, non-hygroscopic, relatively water soluble (23 g L^{-1} at 13°C) and chemically and physically stable in normal environment conditions (Prasad *et al.*, 1971). These properties allow DCD to be effectively formulated with a wide variety of N fertilizers, including ammonium salts. McCarty and Bremner (1989) established that the inhibitor DCD declines in efficacy as soil temperature increases from 10°C to 30°C . DCD inhibits the cytochrome oxidase that is responsible for ammonium oxidation by *Nitrosomonas*. The decomposition products associated with DCD, guanilurea and guanidine have no effect on nitrification, which finally were converted in urea, a conventional fertilizer (Amberger, 1991). Commercial N fertilizers formulated with DCD contain between 5% and 15% DCD-N (Reeves *et al.*, 1986).

On the other hand, DMPP (3,4-dimethylpyrazole phosphate), a nitrification inhibitor developed by BASF (Limburgerhof Research Centre, Germany), also inhibits only the first stage of nitrification, reducing the rate of conversion of NH_4^+ to NO_2^- (Serna *et al.*, 1994). DMPP can be added to either conventional fertilizers or to slurries; it is highly specific in its action, and only a small amount (0.8% of applied nitrogen) is needed

to inhibit nitrification for several weeks. Zerulla *et al.* (2001) have referred to the physical and chemical properties of DMPP. These authors and Díez *et al.* (2008) have proved that DMPP reduces nitrate lixiviation, though the duration of its action depends on climatic conditions, and particularly temperature and humidity (Pasda *et al.*, 2001). DMPP remains effective in the upper soil layer even after heavy rainfall due to its low solubility (Fettweis *et al.*, 2001). DMPP has passed all of the toxicological and ecotoxicological tests to which it has been subjected (Roll, 1999) and has proved to be highly plant compatible (Zerulla *et al.*, 2001). In general, as proved by Barth *et al.* (2001), nitrification inhibitors such as DMPP are more effective in soil with coarse texture.

The results obtained with nitrification inhibitors have, however, been contradictory, probably because there are many factors that affect their soil efficiency, such as organic carbon content, temperature, irrigation rate, and the possible toxicity of these compounds or associated ammonium accumulation (Reeves and Touchton, 1986). One factor that has contributed to these contradictory results has been the uses of unsuitable techniques for measuring the amount of nitrate leached (Starr and Paltineanu, 1998). Few recently published studies treat this subject with reference to Mediterranean agriculture (Serna *et al.*, 1994).

In the case of lixiviation, drainage is considered one of the main factors determining nitrate leaching, which is difficult to measure with undisturbed soil conditions (Webster *et al.*, 1993). However, indirect methods based on a detailed knowledge of local soil water dynamics can improve our ability to estimate drainage from cropping systems (Vázquez *et al.*, 2005).

The main aim of the experiments reported in this paper was to compare DCD and DMPP as inhibitors of ammonium oxidation and nitrate leaching after applying fertilizer to a maize crop grown under Mediterranean conditions. A secondary aim was to compare the relative effects associated with different nitrification inhibitors and split applications of nitrogen on maize yield and nitrate leaching.

Material and methods

Experimental design and crop management

The experimental site was located at the La Poveda Field Station in Arganda del Rey (Madrid) ($40^\circ 19' \text{N}$,

Table 1. Physicochemical properties of the top soil before sowing

Descriptor	EUF ¹ (mg kg ⁻¹)		
pH _{H2O}	8.1 ± 0.1 ²	P 20°C ³	0.14 ± 0.02
OM ⁴ (g kg ⁻¹)	14.0 ± 0.2	K 20°C	1.22 ± 0.21
CaCO ₃ (g kg ⁻¹)	34.0 ± 0.8	N (20°C + 80°C)	0.83 ± 0.15
Texture	Sandy loam	Ca 20°C	3.90 ± 0.24
Bulk density (Mg m ⁻³)	1.47		

¹ Electroultrafiltration technique (Nemeth, 1979). ² Standard deviation. ³ Fraction I obtained at 20°C, 200 V and 50 mA. Fraction II obtained at 80°C, 400 V and 150 mA. ⁴ Organic matter.

3°19'W), in the Jarama River basin. The soil, a *Typic Xerofluvent* (Soil Survey Staff, 1993), was a sandy-loam that became progressively sandier with depth and had a gravel layer at a depth of 1.5 to 2.2 m. Physicochemical characteristics in the top 0–50 cm of the soil profile are shown in Table 1. Soil samples were analyzed for pH, organic matter (Walkley and Black, 1934) and carbonate (ISO 10693, 1995). Nitrogen, P, K and Ca levels were estimated using the electroultrafiltration (EUF) technique (Nemeth, 1979). Total N was determined from EUF extracts (EUF-N) from soil samples by digestion with UV radiation and subsequent oxidation with potassium persulphate in an alkaline medium (Díez, 1988). The phosphorus level was determined also colourimetrically using ammonium molybdate as a reagent (AOAC, 1990). Potassium and Ca levels were determined by flame emission photometry (AOAC, 1990).

The basic fertilizer used in all the treatments was ammonium sulphate nitrate (ASN; 26% N, 19.5% N-NH₄⁺ and 6.5% N-NO₃⁻) a traditional N source, when DCD or DMPP is used as a nitrification inhibitor. ASN-DCD contains 26% total N, of which 5% is DCD. For our study, this fertilizer was prepared by Fertiberia S.A. (Madrid, Spain) from a mixture of the two products, adding liquid vaseline (2% w/w) as an adhesive to improve its stability. It was prepared 10 days before application. In the case of ASN-DMPP, ENTEC (trade mark) was used, which is a commercial fertilizer manufactured by COMPO (0.8% N content) (Barcelona, Spain).

The experimental area included fifteen 100-m² plots. A completely randomized design (CRD), with 5 treatment and 3 replications, was used during the first and second year. The treatments included a control without fertilization (C), a single application of ASN (ASN), a split application of ASN (SP), a single N application with ASN-DCD 5% (DCD) formulated for us, and a single N application with ASN-DMPP (DMPP) commercially prepared. In the second year, the plots

received the same treatments as in the first, but with modified doses of N fertilizer (see doses of nitrogen).

Maize (*Zea mays* cv. *Helen*) cycle 700 (Syngenta) was grown at the experimental site in 2006 and 2007. It was sown the beginning of April in both years. The rows were spaced 75 cm apart, and the plant density was 90,000 plants ha⁻¹. During seedbed preparation, super-phosphate (18% P₂O₅) and K₂SO₄ (50% K₂O) were applied at 22 kg P ha⁻¹ and 111 kg K ha⁻¹, respectively. The maize was grown using traditional farm practices (INFOAGRO, 2009) for the area and was harvested on 6 October 2006 and October 18, 2007, when the grain was mature. In May, the experimental plots were weeded manually.

Maize plants were harvested from the central 10 m of the rows in each plot, and aboveground biomass was determined. Ten harvested plants were selected randomly before their different parts (stalks, leaves, bracts, cobs and grain) were separated, weighed, oven-dried for 24 h at 60°C, and 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 a percentage of grain weight over aboveground biomass. Grain yield was calculated by multiplying aboveground biomass by the harvest index and expressed on a 14% dry matter basis. Nitrogen concentration was determined in plant fractions using a Kjeldhal method (AOAC, 1990) and pretreated with a solution of salicylic acid and sulphuric acid (Bremner, 1965). Plant N accumulation was calculated by multiplying fraction yields by their respective N concentrations.

Soil available nitrogen and doses of nitrogen

Topsoil samples (to a depth of 0.30 m) were taken in the 15 plots and in each season, which was done before planting the maize by use of a soil-core sampler. Available N was calculated by applying a EUF method

(Nemeth, 1979) (Vogel S-724) having previously calibrated organic EUF-N with respect to the amount of potentially mineralized nitrogen in the soil according to its N balance (Sánchez *et al.*, 1998). Available N included mineral N plus potentially mineralized N (N available calculated was 86 and 125 kg N ha⁻¹ in 2006 and 2007, respectively). The doses of N fertilizer were calculated according to nitrogen requirements and considering the nitrogen available in the soil.

The treatments were applied at a dose of 220 kg N ha⁻¹ in 2006 and 180 kg N ha⁻¹ in 2007, for extraction of N provided by the cultivation of 240 kg N ha⁻¹. They were applied only once: on June 1, 2006 and on June 7, 2007, after sowing, as top-dressing when plants had six leaves. In the SP treatment, the ASN fertilizer was split into two applications: one third was applied during seedbed preparation (70 kg N ha⁻¹ in each year) and the remaining two thirds (150 and 110 kg N ha⁻¹ in 2006 and 2007, respectively) were applied on the same date as the rest of the fertilizers.

Monitoring soil mineral N

Soil samples were taken at depth of 0-0.30 m in each plot after the fertilization, once a week during two months, in 2006 and 2007. Twenty sub-samples were combined into one sample per plot at each sampling. The samples were air dried, extracted with 1 M KCl at a ratio of 1:5 parts by volume to weight-KCl solution to soil, centrifuged and decanted and a portion of supernatant were stored in a freezer for subsequent analysis. NO₃⁻-N concentration was determined colourimetrically using a Technicon AAII Auto analyzer with N1 naphthylethylenediamine (Strickland and Parsons, 1968). The ammonium content was analyzed using ion-selective electrodes (Orion Research AG, USA).

Monitoring soil water content and drainage

The water used throughout the experiment was taken from an irrigation channel fed by the River Jarama. This water was sampled 18 times in the course of the experiment to determine the quality components of the irrigation water. An overhead mobile-line sprinkler system was used to irrigate the maize. Irrigation started on 19 June, 2006 and on 9 June, 2007 and continued until the end of August. The maize was watered every 7 to 10 days following the schedule traditionally (INFOAGRO, 2009) used by most growers in the area

(although we applied lower water rate than their). The depth of the water table fluctuated from 4 to 4.5 m below the soil surface, depending on rainfall and river discharge. The average rainfall in this area is 460 mm yr⁻¹.

Drainage at a depth of 1.5 m was calculated by applying the following water balance equation for each measurement:

$$D = R + I - ET_c \pm \Delta S \quad [1]$$

where: D is the drainage (mm), R is the rainfall (mm), I is the irrigation (mm), ET_c is the crop evapotranspiration (mm) and ΔS is the observed change in soil water reserves (mm) from depths of 0 to 150 cm. The water storage in each layer was obtained as the product of the θ_v (m³ m⁻³), which was measured in each layer using capacitance probes and multiplied by the thickness of the layer (m) in question.

Crop evapotranspiration (ET_c, Allen *et al.*, 1998) was estimated from the equation:

$$ET_c = ET_o * K_c \quad [2]$$

where ET_o = reference crop evapotranspiration (mm d⁻¹), estimated from meteorological data according to the FAO Penman-Monteith equation; K_c = crop coefficient (Allen *et al.*, 1998) (dimensionless) during the different phenological stages of the crop. Meteorological data were collected with a Vantage Pro Plus weather station (Davis Instruments, Hayward, California, USA) placed next to the plots. A data logger recorded data on an hourly basis (Fig. 1).

Four (50 mm inside diameter) EnviroSCAN probes (Sentek Pty Ltd, South Australia) were positioned at a depth of 1.5 m. in 4 plots corresponding to the Control, DCD, DMPP, and SP treatments to monitor volumetric soil water content (θ_v). Five capacitance sensors using frequency domain reflectometry (FDR) (Fares and Alva, 2000) were installed in each probe to measure θ_v at depths of 10, 40, 70, 120, and 150 cm. The capacitance sensors, which had previously been mounted inside the probes on pieces of plastic in order to place them at the specified soil depths, were then inserted into previously installed PVC tubes, and connected by wire to the data logger. This installation method prevented the formation of air pockets around the tube and caused minimal disturbance to the soil (Starr and Paltineanu, 1998). A comparison between EnviroSCAN and other measuring devices is reported by Paltineanu and Starr (1997). The frequency signal (FS) from the device was converted into a percentage of volumetric water (θ_v) using a normalization equation based on frequency readings from the sensor when

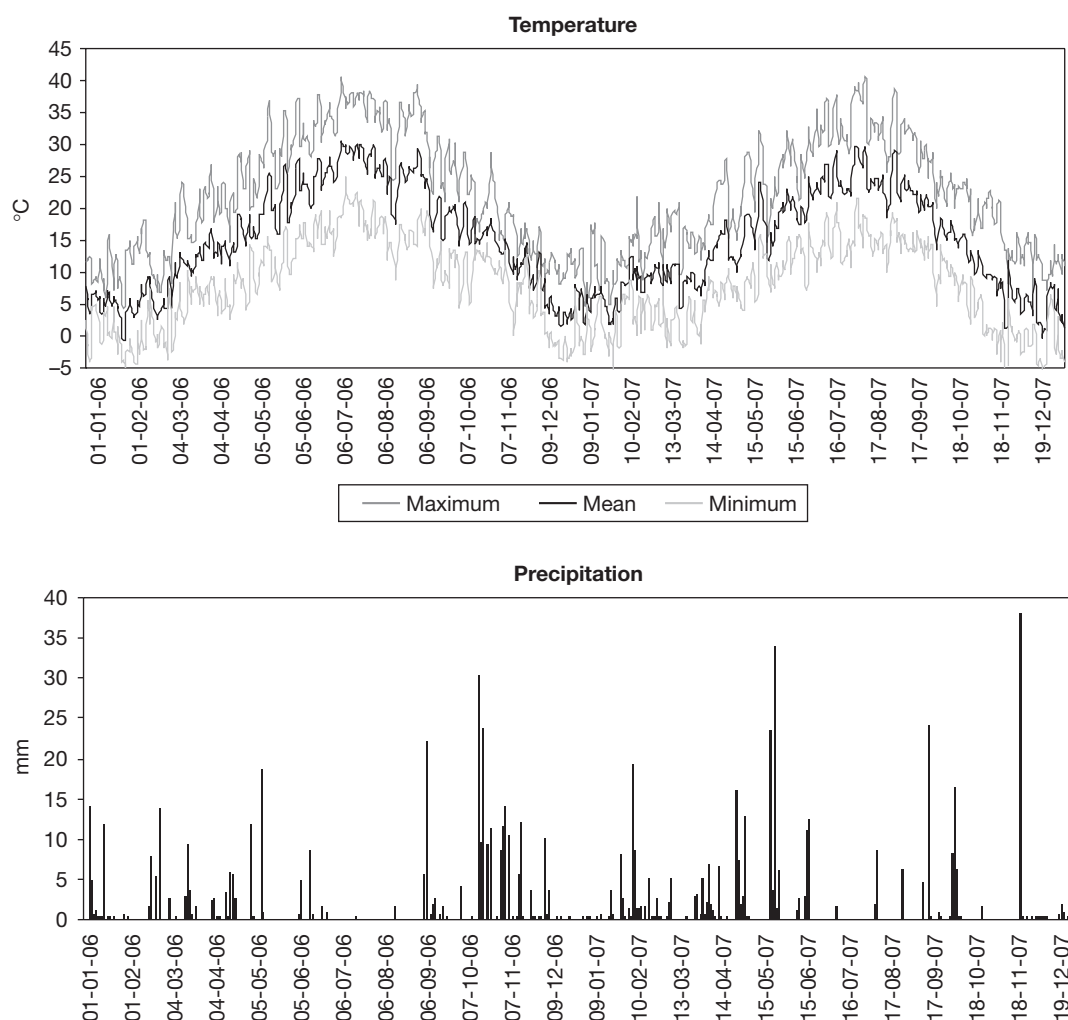


Figure 1. Maximum, minimum and mean temperatures and precipitation for 2006 and 2007.

exposed to air and water. The equipment was specifically calibrated for the soil in question, using the calibration equation proposed by Paltineanu and Starr (1997). The device was programmed to take one reading every hour throughout the cultivation periods in both years. A data logger recorded the data.

Drainage was calculated as the mean drainage for each of the four plots (20 measurements: 4 probes \times 5 depths). Figure 2 shows the data for cumulative drainage obtained in 2006 and 2007.

Nitrate leaching

A ceramic candle extraction system was used to obtain samples of the soil solution (interstitial water). This involved installing two tubes at a depth of 1.4 m in each plot (Díez *et al.*, 1997). These depths were determined after previously estimating the particle size

distribution within the soil profile and the heterogeneity with depth of the gravel layers in the different plots (Díez *et al.*, 2000). We considered that any water reaching this level, near the gravel layer, had been leached to the groundwater (at an average depth of 4 m) because of the high hydraulic conductivity (Smith and Mullins, 1991). Consequently, the amount of drainage water at a soil depth of 1.4 m was the same as that at greater depths due the textural characteristics of the soil profile. Water samples extracted using the ceramic candle were assumed to represent the nitrate concentration of the drainage water.

The ceramic candle was fitted with (63 mm inside diameter, 7 cm long) porous ceramic cups (Nardeux Humisol, Les Ulis, France). The soil solution was collected, on a monthly basis, by means of an electric vacuum pump connected to a nylon tube and was then transferred to a storage bottle. A -80 kPa vacuum was applied

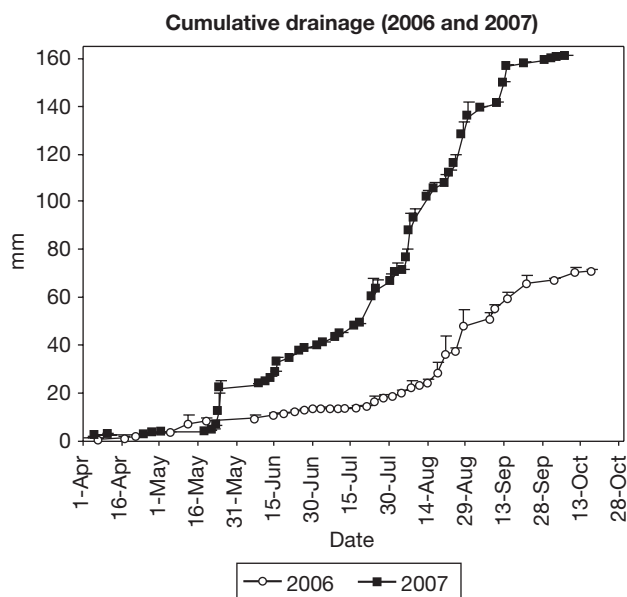


Figure 2. Cumulative drainage in 2006 and 2007. The vertical lines indicate error bars for each measurement.

to the tubes and maintained for a period of 7 to 10 days at each sampling. After this period, water samples were extracted from inside of tube, using air pressure.

Samples of the soil solution were extracted 9 times in both 2006 and 2007, in both cases during the crop periods and NO_3^- , NH_4^+ concentration, and EC were determined. During drainage periods, NO_3^- leaching was calculated on a weekly basis by multiplying the weekly drainage time by the corresponding NO_3^- con-

centration at 1.4 m for each sampling event (Díez *et al.*, 1997). Nitrate and ammonium concentrations were determined following the same procedures as previously mentioned.

Statistical analysis

Two-factor analyses of variance (ANOVAs) [Statgraphics Plus 5.1 (Manugistics, 2000)] were used to examine differences between treatments (with five levels: C, DCD, DMPP, ASN and SP) and the cropping periods (with two levels: years 2006 and 2007) with respect to the variables: nitrate concentration in the soil solution, dry matter, grain yield and plant N accumulated. ANOVAs were performed with a 0.01 α -level. Duncan's multiple range tests (Duncan, 1955) were used to compare differences between treatments.

Results

Soil nitrogen

The changes in mineral N over time determined from soil extracted with 1M KCl in the 2006 and 2007 seasons are shown in Figure 3 (ammonium) and Figure 4 (nitrate). In both years, higher ammonium values were observed after fertilization in treatments including a nitrification inhibitor. Fifteen and 20 to 27 days after N application, in 2006 and 2007 respectively, soil NH_4^+

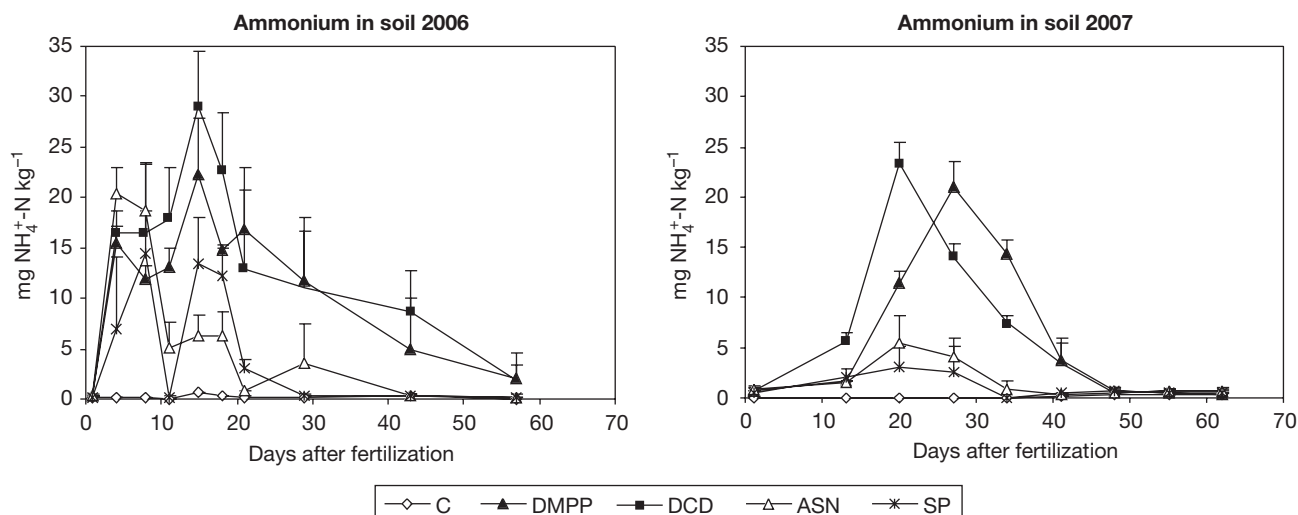


Figure 3. NH_4^+ extracted from top soil by 1M KCl in 2006 and 2007, shown in days after N fertilization. Values are means of three replicates. Treatments: C, DCD, DMPP, ASN and SP respectively refer to the unfertilized control, ASN-DCD 5%, ASN-DMPP 0.8%, ammonium sulphate nitrate and ASN split at optimal rates of N application. The vertical lines indicate error bars.

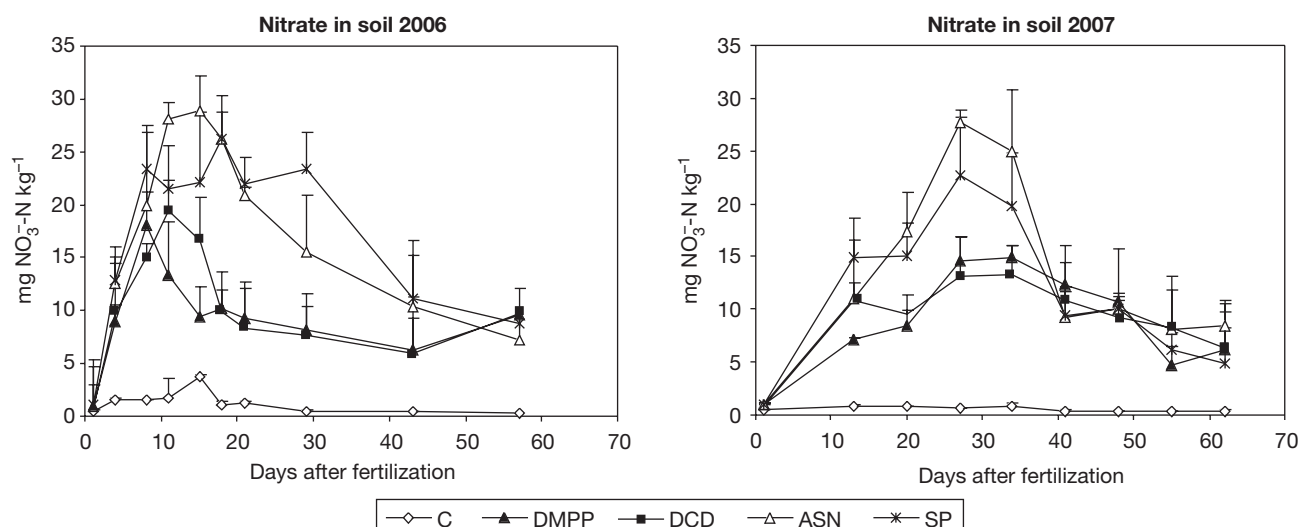


Figure 4. NO_3^- extracted from top soil by 1M KCl in 2006 and 2007, shown in days after N fertilization with treatments C, DCD, DMPP, ASN and SP. Values are means of three replicates. The vertical lines indicate error bars.

concentrations reached their highest values in treatments with nitrification inhibitors: 29 and 22 mg $\text{NH}_4^+\text{-N kg}^{-1}$, in the DCD and DMPP treatments, respectively, in 2006; and 23 and 21 mg $\text{NH}_4^+\text{-N kg}^{-1}$ in the DCD and DMPP treatments, respectively, in 2007. ANOVA showed significant differences between the control no fertilized and the rest of the treatments ($P < 0.01$) and among days after fertilization ($P < 0.01$), but not between replications.

In contrast, nitrate levels (Fig. 4) increased significantly in the first 15 and 27 days after fertilizer application in treatments without a nitrification inhibitor up to 29 and 28 mg $\text{NO}_3\text{-N kg}^{-1}$ in 2006 and 2007, respectively. The treatments with DCD or DMPP reached their highest soil nitrate concentration of 19 (DCD) and 18 (DMPP) mg $\text{NO}_3\text{-N kg}^{-1}$ in 2006, and 13 (DCD) and 14 (DMPP) mg $\text{NO}_3\text{-N kg}^{-1}$ in 2007. The ANOVA for nitrate showed significant differences between the control no fertilized and the rest of the treatments ($P < 0.01$) in both years. However, no significant differences were observed between the ASN and SP and between the DMPP and DCD treatments, in either season. Significant differences were observed between days after fertilization ($P < 0.01$), but not between replications.

Contribution of nitrogen through water irrigation

The average quality components of the irrigation water were: NO_3^- , 9.24 ± 4.4 mg N L^{-1} ; Na, 90 ± 16 mg L^{-1} ; total solids, 650 ± 50 mg L^{-1} ; EC, 1.0 ± 0.1 dS m^{-1} ;

Na adsorption ratio (SAR), 1.55 ; and pH, 7.6 ± 0.2 . These values show the excellent quality of the water used for the experiment.

Under our experimental conditions, 80% of the water applied during the two year study was used by the crop, although drainage was greater in 2007 (161 mm) than in 2006 (71 mm), due to different irrigation frequencies and particularly the rainfall pattern. Similar ET values were obtained in 2006 (782 mm) and 2007 (810 mm). The amounts of irrigation water applied to the maize crops in 2006 and 2007 were 788 and 778 mm, respectively. On the other hand, the mean nitrate concentration in irrigation water was 12.7 mg $\text{NO}_3\text{-N L}^{-1}$ in 2006 and 5.7 mg $\text{NO}_3\text{-N L}^{-1}$ in 2007. Consequently, the N contributions through irrigation water were 94 and 45 kg N ha^{-1} in 2006 and 2007, respectively. The water balances during the crop-growing periods of both years have been included in Table 2.

Drainage and nitrate leaching

The water lost due to drainage represented an average equivalent to 10 to 20% of the total irrigation water

Table 2. Rainfall (mm), irrigation (mm), ET_c (crop evapotranspiration, mm) and drainage (mm), during the crop-growing season

Year	Rainfall	Irrigation	ET_c	Drainage
2006	100	788	782	71
2007	293	778	810	161

Table 3. Mean nitrate concentration (in mg NO₃⁻ L⁻¹) in the soil solution for each treatment, at 1.40 m depth during 2006 and 2007. Duncan's tests show significant differences between treatments within each year

Seasons ^b	C ¹	DCD	DMPP	ASN	SP
2006	15.1a	76.2c	56.1b	113.0d	100.0cd
2007	34.3a	143.5b	149.9b	209.6c	285.0d

¹ Data based on six replicate ceramic candle extractions taken at a depth of 1.4 m (9 samplings per growing season). Means followed by different letters for each row indicate significant differences between treatments. C: control no fertilized. DCD: 5% ASN-DCD. DMPP: 0.8% ASN-DMPP. ASN: ammonium sulphate nitrate. SP: ASN split ($P < 0.01$, Duncan test).

applied. In 2007, the drainage loss was great due to intense rain (293 mm), whereas in 2006 loss was only 100 mm, favouring the tests to establish the effect of the nitrification inhibitors on NO₃⁻ leached under conditions of higher drainage. The differences observed between water inputs and losses in the system were attributable to the water reserves present in the soil before previous to the crop period.

Significant differences were obtained between seasons ($P < 0.01$) and among treatments ($P < 0.01$) (Table 3). The nitrate concentrations at a depth of 1.4 m obtained in 2007 were generally greater than those for 2006. Duncan's test shows significant differences between treatments within each year (Table 3). Although in 2007 there were no significant differences between the DCD and DMPP treatments, in 2006, the DCD treatment showed significant differences from DMPP. The higher

nitrate concentrations were obtained in the without inhibitor treatments (ASN and SP).

Due to low drainage in 2006, the nitrate leached in all treatments was very low, as Figure 5 shows, and the differences observed between treatments were very small. However, when N losses are high due to high drainage, as occurred in our 2007 experiment, a greater response to DMPP and DCD would be expected. Figure 5 shows the greatest losses due to NO₃⁻ leaching occurred in 2007 and also that there were appreciable differences among treatments, especially between those involving nitrification inhibitors (which exhibited smaller losses) and those involving ASN alone. DCD and DMPP treatments, obtained in 2007, showed similar performances (54.7 and 53.1 kg N ha⁻¹ with DCD and DMPP, respectively). The treatments C and ASN showed clear differences with values of nitrate leaching of 12 and 78 kg N ha⁻¹, respectively.

Plant N accumulation and grain yield

In general, grain productions were higher in 2007 than in 2006 no doubt because of more moderate climatic conditions. Figure 1 shows maximum temperatures of 36°, 37° and 41°C respectively observed in May, June and July, 2006, while in 2007, the maximum temperatures were 32°, 33° and 40°C in the same months. But the lowest maximum temperatures in 2006 were 20°, 26°, and 34°C, registered in May, June, and July, respectively; whereas in 2007, the equivalent registers

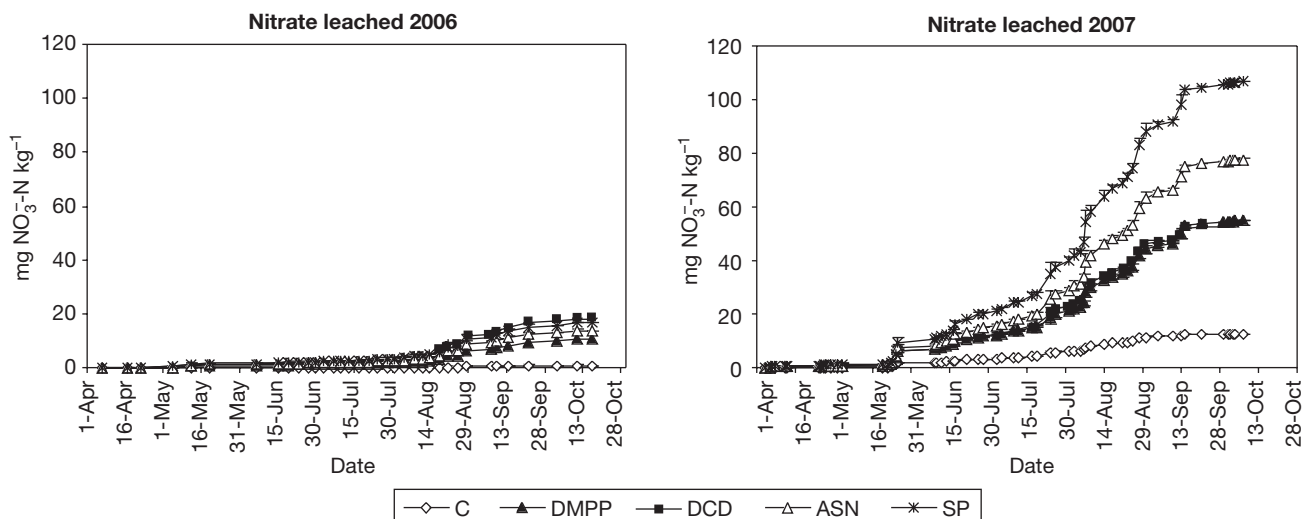


Figure 5. Nitrate leaching in 2006 and 2007 during the maize crop, according to treatment (C, DCD, DMPP, ASN and SP). Values are means of four drainage replicates and six nitrate concentration replicates. The vertical lines indicate error bars.

Table 4. Mean of dry matter (kg ha⁻¹), grain yield (kg ha⁻¹) and plant N accumulation (kg ha⁻¹) during 2006 and 2007. Duncan's tests show significant differences between treatments within each year

Growing seasons	Treatments ¹	Dry matter	Grain yield	Plant N accumulation
2006	C	10,918a ²	6,955a	78.5a
	DMPP	16,776b	11,935b	163.0b
	DCD	18,807bc	13,520bc	184.3bc
	ASN	19,353bc	13,515bc	176.6bc
	SP	21,619c	15,207c	212.8c
2007	C	17,272a	9,714a	133.7a
	DMPP	25,502b	15,239b	219.9b
	DCD	27,138b	16,102b	254.9b
	ASN	26,975b	16,099b	266.2b
	SP	26,887b	15,776b	260.4b

¹ C, DCD, DMPP, ASN and SP denote the treatments: unfertilized control and no inhibitor, ASN-5% DCD, ASN-0.8% DMPP, ammonium sulphate nitrate and ASN split at 220 kg N ha⁻¹ in 2006 and 180 kg N ha⁻¹ in 2007. ² Different letters in a given column for a given year indicate significant differences between treatments ($P < 0.01$).

were 15°, 22° and 29°C. Also, it must be regarded that May rainfall was higher in 2007 than 2006.

Significant differences in dry matter, plant N accumulation and grain yield were observed between seasons and treatments ($P < 0.01$). The Duncan multiple range tests showed only significant differences between treatment C (no fertilized) and the rest of the treatments in 2007. However, in 2006, significant differences were observed not only with respect to the treatment C but also between fertilized treatments, and especially between DMPP and SP (Table 4).

Discussion

Soil analysis carried out after fertilization showed an increase of ammonium and a reduction of nitrate content in the treatments with nitrification inhibitors. The ammonium content in 2006 was greater than in 2007 (Fig. 3); this was probably due to higher air temperatures during May and June 2006 and also to lower level of rainfall (31 mm in 2006 as opposed to 134 mm in 2007). The higher rainfall during May and June 2007 could explain the lower values in soil ammonium and nitrate contents observed in June.

Soil solution nitrate concentrations were affected by the different treatments (Table 3). In general, drainage water from fertilized plots contained high NO₃⁻ concentrations (between 2.2 and 435 mg NO₃⁻ L⁻¹ in 2006 and between 10 and 950 mg NO₃⁻ L⁻¹ in 2007) and very low

NH₄⁺ concentrations (between 0 and 0.37 mg NH₄⁺ L⁻¹ in 2006 and between 0 and 0.26 mg NH₄⁺ L⁻¹). The average concentrations of nitrate in drainage water were lower in treatments with inhibitor (DMPP and DCD) (Table 3). No significant differences occurred between DMPP and DCD in 2007. Either DCD or DMPP lengthened ammonium presence in soil in a similar manner and showed lower NO₃⁻ concentrations (30%) than in the control plots fertilized with ASN.

Data on nitrate concentrations for the soil solution at a depth of 1.4 m were used to study the possibility of groundwater pollution. Cumulative NO₃⁻ discharge at a depth of 1.4 m depended mainly on the irrigation water and fertilizer treatment applied. The poor results obtained in 2006 in terms of leached nitrate were due to low drainage (71 mm). These results are similar to obtained by Díez *et al.* (2000) who observed, total leaching depended mainly on drainage and to a lesser extent on variations in NO₃⁻ concentration at the percolation depth.

In 2007, the drainage was greater than in 2006 because the rainfall was higher, and moreover, the frequency of irrigation was modified (leaving only one day between water applications). These changes resulted in a greater value of drainage (161 mm) with similar doses of irrigation and, consequently, the differences between treatments were clearer. Figure 5 shows the marked difference in nitrate leaching between the two seasons.

The results obtained relating on leaching, which show that treatments involving nitrification inhibitors

exhibited a clear tendency to reduce nitrate pollution from leaching. In the 2007 experiment, the use of N-containing fertilizers plus DMPP or DCD reduced nitrate leaching losses of up to 29% ($P < 0.05$) with respect ASN treatment.

The nitrate concentrations and levels of nitrate leaching observed with the SP split were higher than if ASN was applied in a single top dress; this was possibly due to the rainfall in May, particularly in 2007, which dragged a part of fertilizer. The May rainfall obviously did not affect the rest of the treatments in which fertilizers were applied in June.

Previously published results relating to the use of DCD are somewhat contradictory. Some authors, such as Williamson *et al.* (1998), attribute only very minor effects to DCD in terms of reducing nitrate leaching, but the doses used in their experiments were very low (1.1% DCD). Similarly, Davies and Williams (1995), working with a soil column, did not observe any significant effects with DCD. However, other authors have reported similar results to those reported by us. For example, Francis *et al.* (1995) and Cookson and Cornforth (2002) reported that DCD was effective for reducing nitrification and that it consequently reduced leaching. Serna *et al.* (1994) observed that if they applied DCD at 2%, nitrate leaching was reduced, with the loss of only 20% of the N added with this treatment as opposed to 68% without DCD. These authors (Serna *et al.*, 2000) concluded that nitrate concentrations in drainage waters were reduced with DMPP (68% and 53% of the applied N was leached to below 0.60 m in the ASN and ASN + DMPP treatments, respectively). Furthermore, Chaves *et al.* (2006) concluded that, under favourable conditions, DCD is able to inhibit nitrification from cauliflower crop residues for 50 days and DMPP is able to do the same for at least 95 days. Our findings are consistent with those obtained by Irigoyen *et al.* (2003) who established that either DCD or DMPP extended the presence of ammonium in soil.

The SP split treatment did not offer any advantages with respect to nitrate leaching, either with the use of single ASN or compared to the use of nitrification inhibitors. SP was associated with greater losses due to leaching (106 kg N ha⁻¹ in 2007) than ASN (78 kg N ha⁻¹). Authors have compared the effects of nitrification inhibitors vs split N applications (Boswel *et al.*, 1976) with different results. Arregui and Quemada (2006) concluded that applying N fertilizer at rates that were not excessive, neither splitting N fertilizer application nor the use of a nitrification inhibitor, consistently re-

duced nitrate leaching. However, Molina and Ortega (2006) established that the larger N-NO₃⁻ leaching losses associated with the use of fertilizers without a nitrification inhibitor were restricted by split N applications.

Significant differences in ANOVAs, were obtained between years and between fertilized treatments with respect to dry matter, grain yield, and N accumulation ($P < 0.01$). In 2006, the Duncan test (Table 4) shows significant differences between the DMPP and SP treatments. In 2007, only significant differences were obtained between C no fertilized and the fertilized treatments. The results obtained in this paper show that the treatments with nitrification inhibitors did not increase the grain yield but neither did they reduce maize yields. However, some authors have reported positive effects on yield. Molina and Ortega (2006), working with Chilean soils in a ryegrass experiment, established that ASN + DMPP increased dry matter production and the efficiency of N use, and that leaching losses were reduced. Leaf N levels were also higher in plants fertilized with ASN + DMPP (Serna *et al.*, 2000). Other authors, such as Reddy (1964), established that plant toxicity of the nitrification inhibitors was expressed by a reduction in the number of chloroplasts per cell and observed toxicity symptoms in plant at doses of 6 to 7 ppm N-DCD. However, the same author emphasizes that maize, wheat, or oats moderately tolerate DCD at rates of between 6 and 17 ppm of N-DCD. Our results were similar to those obtained by Reeves and Touchton (1986) who reported that commercial N fertilizers formulated with DCD contain between 5 and 15% DCD-N produced no observable signs of toxicity.

The drainage rate was the most important component of nitrate leaching. The experiment of 2006 showed that the low drainage rate resulted in a sharp decline of nitrate leaching. However, the experiment of 2007 showed clear differences in nitrate leaching between treatments due to the greater drainage. The applying of 5% DCD or 0.8% DMPP had the positive effect of reducing nitrate pollution. Either nitrification inhibitors lengthened ammonium presence in soil in a similar manner. In 2007, soil NO₃⁻ concentrations in treatments involving nitrification inhibitors were 30% lower than in the plots fertilized with ASN. In consequence, the use of N-containing fertilizers plus DMPP or DCD reduced nitrate leaching losses of up to 29% ($P < 0.05$) with respect ASN treatment.

Freshly prepared ASN-DCD (see fertilizer in Material and methods), exhibited excellent properties for

controlling nitrate leaching. This was particularly evident if we take into account the fact that grain yields and N accumulation were similar for the DCD and ASN treatments for the same N doses. The use of DCD was associated with higher grain yields than DMPP, no significant differences were apparent. There were no significant differences among fertilizer treatments in terms of maize yield, either with or without nitrification inhibitors; this demonstrates that the DCD or DMPP treatments used in this experiment had no toxicity effects on plants. These results are consistent with those reported by Roll (1999) and Zerulla *et al.* (2001).

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