Fertilizer Research **32**: 1–11, 1992.

Effect of a new nitrification inhibitor (wax coated calcium carbide) on transformations and recovery of fertilizer nitrogen by irrigated wheat

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Received 14 August 1991; accepted in revised form 12 January 1992

Key words: Nitrogen loss, denitrification, acetylene, irrigation, urea, nitrogen isotopes

Abstract

The effectiveness of wax coated calcium carbide to provide a slow release of acetylene to inhibit nitrification and denitrification in soil was evaluated in a field experiment with irrigated wheat (cv. Condor) grown on a red brown earth in the Goulburn-Murray Irrigation Region. The effect of the inhibitor treatments on biomass and grain yield was determined in 25 m × 3 m plots, and the effect on recovery, in the plant-soil system, of urea-N applied at sowing was determined in 0.3 m × 0.3 m microplots using a ^{15}N balance technique. The inhibitor limited ammonium oxidation, prevented nitrogen loss by denitrification for 75 days, increased N accumulation by the wheat plants, increased grain N and resulted in a 46% greater recovery of applied nitrogen in the plant-soil system at harvest. However, the inhibitor treatment did not increase grain yield because of waterlogging at the end of tillering and during stem elongation.

Introduction

Nitrogen fertilizer is an expensive input and in many trials less than 60% of the applied nitrogen (N) is recovered in the crop and soil with the remainder being lost [14, 15, 16]. Increasing the amount of N applied at sowing does not increase the amount of N available to the cereal grain [21, 22], because the fertilizer N is lost before it can be assimilated by the crop. A minimum of six weeks is required before the crop has the capacity or potential to accumulate much of the N applied at sowing [14, 16].

Recovery of urea-N in the crop and soil, when applied at or near heading to irrigated wheat grown on red-brown earths, was higher than that obtained when the fertilizer was applied to the soil at sowing [13, 18]. Between 50 and 70% of the post sowing N applications were recovered in the crops [17, 18] compared to less than 40% when the N was applied at sowing [14, 16]. The fertilizer N is used more efficiently when the supply of available N in the soil is matched with the demand for N by the crop [9].

It has been shown that ammonia volatilization, leaching and runoff were not important mechanisms for N loss from red-brown earths when urea was applied in the irrigation water or broadcast onto the soil and washed in by sprinkler irrigation [16, 19]. The N loss was due principally to denitrification. Loss by denitrification can be prevented by adding compounds to soil to inhibit the oxidation of NH_4^+ by nitrifying organisms [12].

Acetylene has been shown to be a potent inhibitor of nitrification [3, 24]. Concentrations of less than 10 ppm (v/v) are effective in inhibiting nitrification and the effects persist for several days after the acetylene has been removed. However, because acetylene is a gas, there are problems in applying this compound to soil and keeping it there for extended periods to inhibit nitrification. One approach to overcoming these problems is to use calcium carbide as the source of acetylene gas.

$$\underset{\text{Calcium carbide}}{CaC_2} + \underset{\text{Water}}{2H_2O} \rightarrow \underset{\text{Calcium hydroxide}}{Ca(OH)_2} + \underset{\text{Acetylene}}{C_2H_2} \qquad [1]$$

In order for this process to be effective for extended periods, it is necessary to regulate the reaction of calcium carbide with soil water. Banerjee and Mosier [1] found that coating calcium carbide with layers of wax and shellac controlled acetylene production and the nitrification inhibition capacity.

This paper reports the results of a field experiment designed to determine the effectiveness of wax coated calcium carbide as a slow release source of acetylene to inhibit nitrification and thus denitrification of fertilizer N applied to irrigated wheat grown on a red-brown earth.

Materials and methods

The experiments were conducted on a red-brown earth (classification: Dr. 2.33, [10]; *Typic Haplustalf*, [20]) at Tatura, Australia (145°14′ E, 36°24′ S). The profile is characterised by a brown loam A horizon to 0.15 m depth, sharply separated from a red-brown massive clay B horizon to 0.6 m. Some properties of the surface soil are: pH (1:5 soil:water) 6.2; cation exchange capacity, 10.6 cmol [Na^+] kg^{-1}; organic C, 17.8 g kg^{-1}; and total N, 1.6 g kg^{-1}. This soil is typical of a large area (2 million hectares) used for irrigated agriculture in south-eastern Australia. The area was maintained as a clean fallow during the summer months after supporting two consecutive crops of wheat (1987 and 1988). The area had been under lucerne (*Medicago sativa*) in the period 1979 to 1987.

Main experiment

The effect of the inhibitor on yield characteristics was assessed in 25 m × 3 m rectilinear plots with three inhibitor and five N treatments in a split plot design with three replicates. Inhibitor and N treatments were arranged as the main plots, with time of sampling as the subplots. Nitrogen was applied as urea at nil, 5, 10, 15 and 20 g N m^{-2}, and the treatments were designated N_0, N_5, N_{10}, N_{15} and N_{20}, respectively. Wax coated calcium carbide was used to supply acetylene as the nitrification inhibitor. It was applied to give nil, 2 and 4 g CaC_2 m^{-2} and these treatments were designated I_0, I_2 and I_4, respectively.

The wax coated calcium carbide was prepared by placing CaC_2 granules (1–2 mm) in a beaker and mixing in succession with beeswax (40% of CaC_2 by weight), paraffin-vaseline (3:2 by weight; 40% by weight of CaC_2) and finally shellac (10% of CaC_2 by weight). After thorough mixing, the material was dried under a stream of hot air before the next coating material was applied [1].

The area was pre-irrigated in mid-March, cultivated to a depth of 0.20 m, followed by 2 additional tillage operations with a spring-tined cultivator in April. Pre-emergence herbicide 'Yield' (active ingredients 125 g oryzalin L^{-1}, 125 g trifluralin L^{-1} and 220 cm^3 solvent L^{-1}) was applied at 0.18 cm^3 m^{-2} and incorporated with the nitrogen application. The wax coated calcium carbide was mixed with the urea and drilled to a depth of approximately 0.15 m on 3 May 1989, with a cone-seeder fitted with 12 tines each 0.15 m apart. Wheat (*Triticum aestivum* L. *cv*. Condor) was sown with a disc-seeder at 20 g seed m^{-2} in drill rows spaced at 0.15 m immediately after applying the nitrogen. Superphosphate (2 g P m^{-2}) was drilled with the seed. 'Tilt 250 E.C.' was applied 147 days after sowing (147 DAS) to control grass weeds. This was effective and the crop was free of weeds and disease.

Irrigation was by flooding, with the area being totally inundated for approximately 6 hours. Irrigation commenced on 5 October (155 DAS), 5 days before anthesis. Subsequent irrigations were scheduled when the cumulative evaporation (class A pan) minus rainfall deficit was 50 mm. Dates of irrigation and rainfall are shown in Figure 1.

Soil water was measured prior to the first irrigation (152 DAS) and at final harvest (222 DAS) with a neutron probe at 0.10 m intervals from 0.10 to 1.30 m depth. A single access tube was installed in each plot. Moisture content of the surface 0.10 m was measured gravimetrically. Rainfall, minimum and maximum temperature, and Class A pan evaporation were measured

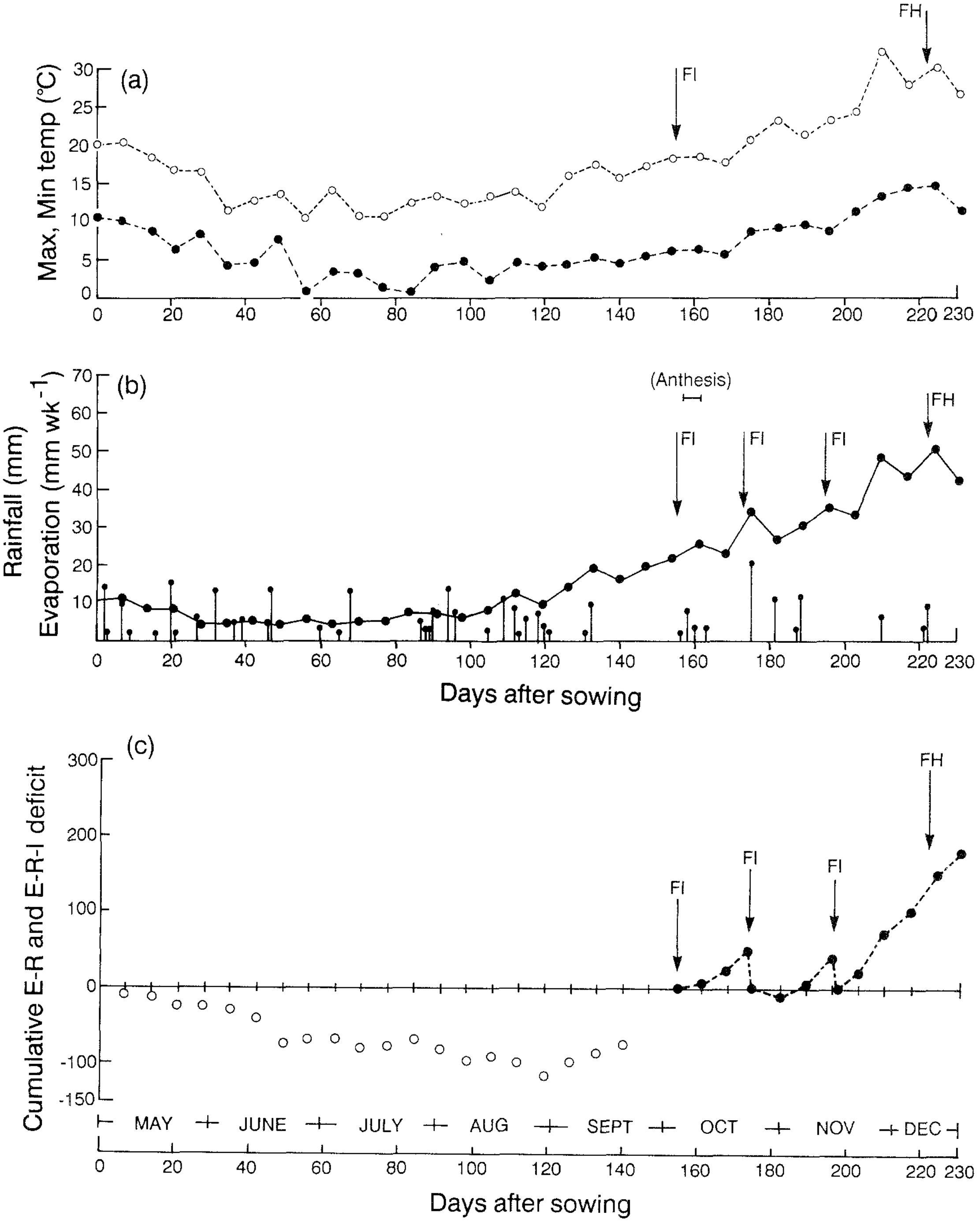

Fig. 1. Seasonal changes in: a) maximum (○) and minimum (●) air temperature; b) rainfall (●), evaporation (-●-); and c) cumulative water deficit from sowing to 140 DAS (○) and from the commencement of irrigation to harvest (●). FI and FH denote irrigation and final harvest, respectively.

daily at the weather station approximately 600 m from the site.

Soil samples

Soil samples were collected from the 0–0.10 m and 0.10–0.20 m depth 2, 6, 8, 12, 20, 26, 34, 41, 54, 69, 84, 97, 140, 169, 198 and 219 days after sowing. On each sampling occasion, 3 adjacent cores (50 mm I.D) were collected between the plant drill rows along a transect perpendicular to the rows. Corresponding depth sections were bulked, and the soil was thoroughly mixed. Moist soil (equivalent to 10 g dry weight) was extracted within 2 h of collection with 0.1 L of 2 *M* KCl solution (Analytical Grade). The soil suspensions were filtered through a Whatman No. 42 filter paper and the filtrate stored at 2°C until analysed for exchangeable ammonium (NH_4^+) and nitrate (NO_3^-) [7]. Gravimetric soil moisture was determined by drying a sub-sample (18–22 g wet weight) for 20 minutes in a microwave oven [6]. Bulk density was determined by the core method using cylinders with an internal diameter of 73 mm [2]. Nitrogen concentrations (g N kg dry soil^{-1}) were converted to an area basis by multiplying by the mass of soil in the respective layers.

Plant measurements

Samples of 1 m of row were taken at approximately 14 day intervals from 58 to 215 DAS; 7 days before the final harvest on 11 December. Plant tops were cut at ground level and a sub-sample (30 tillers) was divided into green leaf, senescent leaf, stem, and head fractions. Leaf area index was calculated from the area of green leaf measured on the sub-sample using an electronic planimeter. The plant material was dried at 60°C for at least 72 h and weighed. The dried material was ground to pass through a 0.42 mm sieve and analysed for total N content.

At the final harvest (222 DAS) yields were determined by cutting the plant tops from a 4.5 m^2 area (6 drill rows by 5 m). Sub-samples were dried at 60°C and analysed for moisture and total N content. The number of spikes in the main sample was counted, and grain yield and individual grain weight were measured after threshing. Grain was oven dried and a subsample retained for total N analysis.

^{15}N Microplot study

The effect of wax coated calcium carbide on the recovery of ^{15}N labelled urea was assessed in microplots with four inhibitor and one N treatment harvested on 8 occasions. There were 5 replicates of each treatment laid out in a randomised block design. The wax coated material was applied to give 0, 2, 4 and 8 g CaC_2 m^{-2}. These treatments were designated I_0, I_2, I_4 and I_8, respectively. One hundred and sixty microplots (0.3 m by 0.3 m and 0.3 m high) were driven 0.28 m into the soil in a non-fertilised area adjacent to the main experiment and the upper 0.15 m of soil was removed from each microplot. Wax coated calcium carbide and a solution of ^{15}N labelled urea (10 g N m^{-2}; 5 atoms % N) was applied in two bands 0.15 m apart thereby simulating the drilling of the inhibitor:fertilizer mixture into the soil. Following the band application of the inhibitor:fertilizer mixture, the surface soil was returned and each microplot sown with 2 rows of wheat. The surrounding area was sown with wheat using the procedure outlined for the main experiment. After crop emergence, the microplots were thinned to 34 plants per microplot; a density equivalent to that of the main experiment.

Twenty microplots were destructively sampled on each sampling occasion, 19, 33, 53, 75, 101, 149, 178, and 215 DAS. The plants were cut at the soil surface, sectioned into 0.01 m pieces and oven dried at 60°C. Dried plant material was ground to pass through a 0.42 mm sieve and analysed for total N and ^{15}N. Following the plant harvest, the entire 0–0.10 m and 0.10–0.20 m soil layers were removed and 2 cores (0.075 m diameter) were taken from the 0.20–0.30 m zone. Soil samples were air dried, weighed and ground to less than 2 mm. Plant crowns and coarse root material, separated from the soil during grinding, were ground to pass through a 0.42 mm screen. The soil, plant crowns and roots were then well mixed and bulk sub-samples finely ground (<250 μm) for total N and ^{15}N analyses.

Chemical analyses

Ammonium in 2 *M* KCl extracts of soil was determined by an indophenol blue method [7], urea was determined by a modified diacetyl monoxime method [8], and nitrite and NO_3^- were determined using an Auto Analyser fitted with a copperized cadmium column by a modified Griess-Ilosvay method [7]. Total N concentration in plant material was determined on an Auto Analyser after micro-Kjeldahl digestion [11]. Total N and ^{15}N enrichment of the plant and soil samples from the ^{15}N labelled microplots were determined by an automated flow method in which Dumas combustion was linked to a mass spectrometer (R.R. Sherlock, Department of Soil Science, Lincoln University, Canterbury, New Zealand; unpublished).

Results and discussion

Weather and soil water

The mean maximum temperature decreased from 20°C to 10°C while the mean minimum temperature ranged from 10°C to 0°C from sowing to mid July. Class A pan evaporation also declined after sowing until 130 DAS, and then increased rapidly during the spring to parallel increases in maximum temperature (Fig. 1). Evaporation increased from 8 mm $week^{-1}$ to 50 mm $week^{-1}$ at the final harvest.

Rainfall from sowing until the first irrigation was 260 mm and cumulative rainfall exceeded cumulative evaporation by 112 mm at 120 DAS (Fig. 1c). The excess water would go into storage in the soil or drain from the laser-levelled field. The upper (UL) and lower limits (LL) to plant available water in the surface 0.70 m were 250 and 140 mm, respectively (Fig. 2). Corresponding values for UL and LL for the depth interval 0.70 to 1.30 m were 225 and 124 mm. At the time of the first irrigation (155 DAS), water storage in the 0.70 to 1.30 m depth was greater than the amount stored at field capacity, whereas between 30 and 56 mm of stored water had been used by the wheat to a depth of 0.70 m. Water use was greatest from treatment N_{20} (Fig. 2). The total soil water deficit at final harvest was only 70 mm to a depth of 1.3 m.

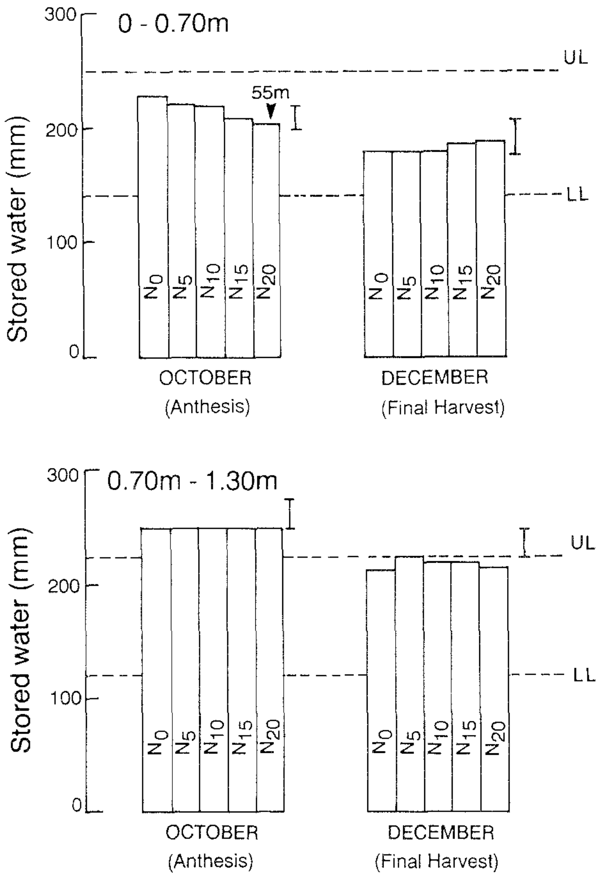

Fig. 2. Effect of N fertilizer on the soil water storage in the 0–0.70 m and 0.70–1.30 m depths near anthesis (152 DAS) and final harvest (210 DAS). UL is the upper limit of water storage when the profile is at field capacity, and LL is the lower limit to plant available water.

Judging from the small changes in the soil water content below 0.5 m between 155 DAS and final harvest (Fig. 3) the crop did not appear to extract water below that depth. Water extraction seemed to be confined to the surface 0.30 m. Soil water contents below 0.25 m, at 155 DAS, were greater than average values measured at field capacity for this site [23]. Similarly, at final harvest water contents below 0.65 m were typical of a wet profile and the inability of the crop to extract water from depth.

Effect of inhibitor on mineral N

Urea-N additions at sowing significantly ($P < 0.001$) increased the NH_4^+ concentration in the soil, especially at the 0.10–0.20 m depth. The NH_4^+ content remained at higher levels for

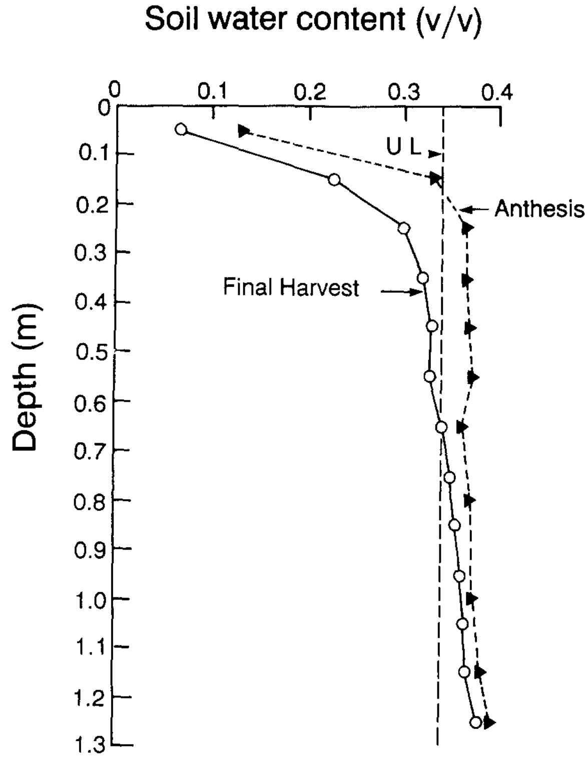

Fig. 3. Mean soil moisture profile for all N fertilizer treatments prior to application of the first irrigation (152 DAS; broken line) and at the final harvest (210 DAS; solid line). UL as in Fig. 2.

longer times as the rate of N increased (data not presented). Ammonium-N concentration was significantly ($P < 0.001$) increased in the 0–0.10 and 0.10–0.20 m depth by treatment I_2 and I_4 (Fig. 4). Values were generally higher in the 0.10–0.20 m layer compared to the surface 0.10 m depth because the fertilizer was drilled to a depth of 0.15 m. Higher NH_4^+ levels were maintained at the 0.10–0.20 m depth during the initial 84 days after sowing. After this time, the NH_4^+ levels in all the N treatments had decreased to an average value (mean over all N treatments) of less than 2 $\mu g\,N\,g^{-1}$ and remained at this low level until harvest.

Nitrate concentrations were significantly ($P < 0.001$) lower in all treatments that received the inhibitor and the difference persisted until 60 DAS (Fig. 4). The lower NO_3^- concentrations in treatment I_2 and I_4 compared to the nil treatment showed that the inhibitor was effective in inhibiting the oxidation of NH_4^+. There was a significant ($P < 0.001$) time by nitrogen by inhibitor interaction on the NO_3^- content in the soil at 0.10–0.20 m depth. The NO_3^- content in the soil at selected times after sowing is shown in Figure 5. Between 8 and 41 DAS, NO_3^- levels increased in direct response to the addition of urea in treatment I_0, but there was no parallel increase in treatments I_2 and I_4. The higher NO_3^- content in treatment I_0 persisted until 84 DAS; after this time there was no difference ($P < 0.05$) in NO_3^- content between all treatments.

The addition of the wax coated calcium carbide had no measurable effect on the pH of the acidic surface soil and thus would not have affected nitrification rates through its effect on soil pH. The wax coating would have ensured that the inhibitor was a slow release source of alkalinity as well as acetylene.

Effect of inhibitor on N accumulation and plant growth

Total N accumulation in the wheat tops increased with time and the addition of inhibitor (Fig. 6). Treatments I_2 and I_4 accumulated a mean of 2.9 $g\,N\,m^{-2}$ whereas treatment I_0 accumulated 2.5 $g\,N\,m^{-2}$. At 58 DAS there was little effect of the inhibitor treatment on N uptake, but by 147 DAS there was a marked effect of the inhibitor on total N accumulation by the wheat plant ($P < 0.05$; Fig. 6). Nitrogen and inhibitor had additive effects on total N accumulation; that is, increasing N and inhibitor applications significantly increased total N accumulation in the aboveground plant (Fig. 6).

Biomass at final harvest was increased by the N addition, and the fitted orthogonal polynomial function had significant linear ($P < 0.001$) and quadratic ($P < 0.001$) terms (Fig. 7). The inhibitor treatment had no effect on the biomass at harvest. Mean biomass production for treatments N_0, N_5, N_{10}, N_{15} and N_{20} were 713, 1131, 1164, 1368 and 1317, respectively. The effect of urea-N addition on grain yield was equivalent to the response on biomass and the fitted response curve also had significant linear ($P < 0.001$) and quadratic ($P < 0.001$) terms. Mean grain yields were 158, 260, 240, 333 and 302 $g\,m^{-2}$ for treatments N_0, N_5, N_{10}, N_{15} and N_{20}, respectively. There was no additive effect of the inhibitor on grain yield.

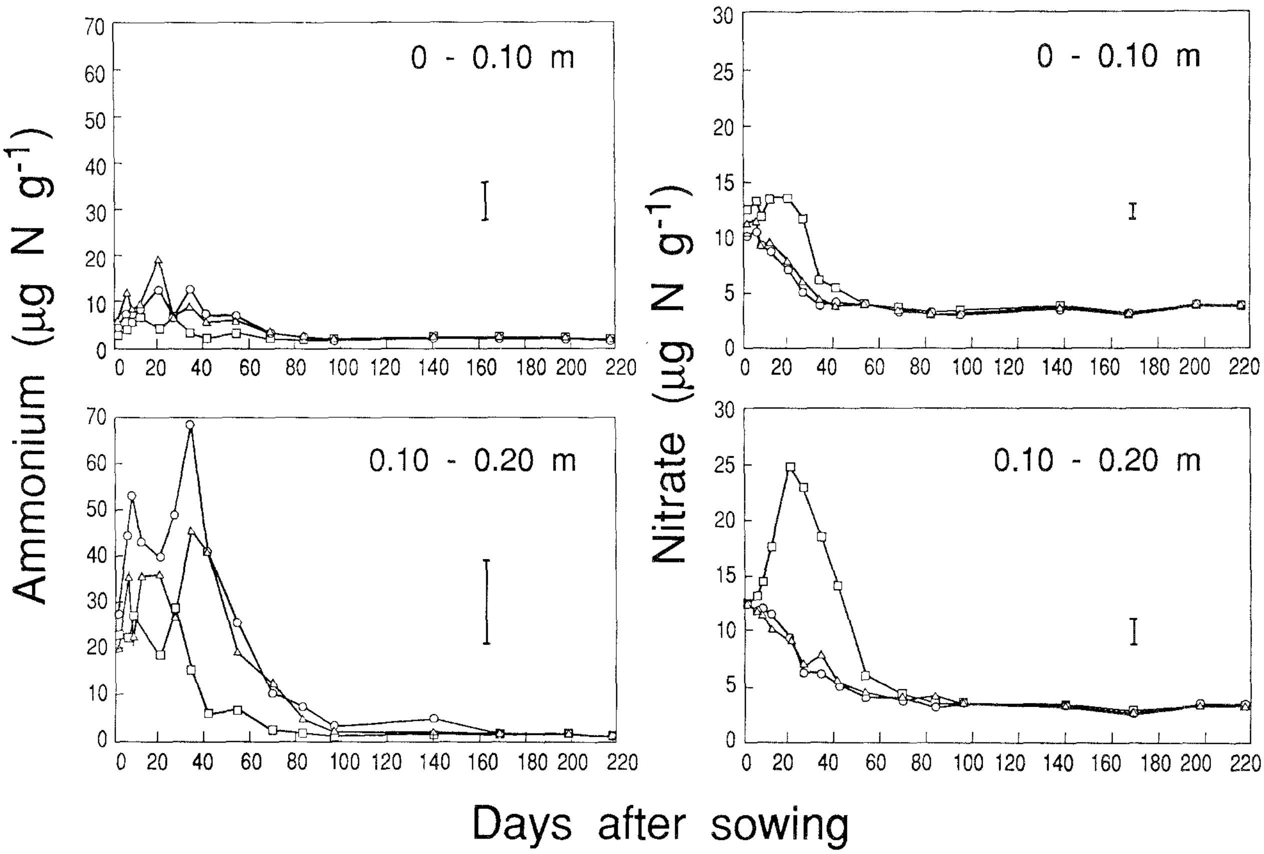

Fig. 4. Effect of inhibitor on extractable mineral N in the 0–0.10 m and 0.10–0.20 m soil layers with time. Treatments I_0 (□), I_2 (△), and I_4 (○). Vertical bars represent the L.S.D. ($P = 0.05$) appropriate to comparisons between time.

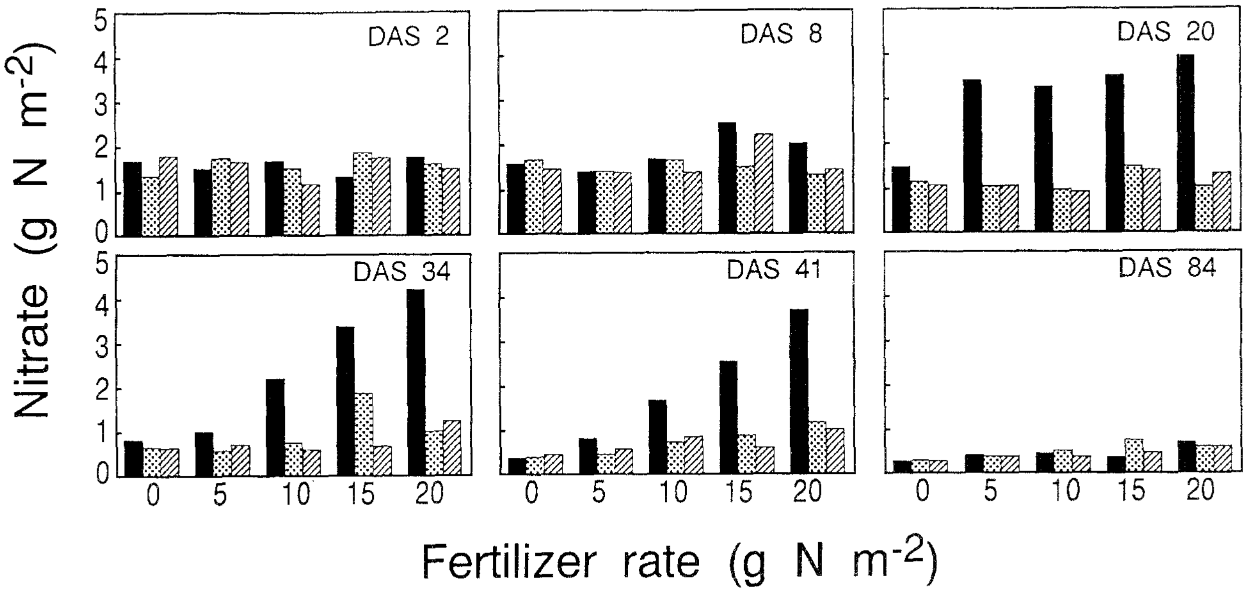

Fig. 5. Effect of inhibitor and N fertilizer rate on KCl extractable nitrate in the 0.10–0.20 m soil layer at selected times. Treatments I_0 (■), I_2 (▩), and I_4 (▨). The L.S.D. ($P = 0.05$) for comparison between time by nitrogen by inhibitor is 0.76.

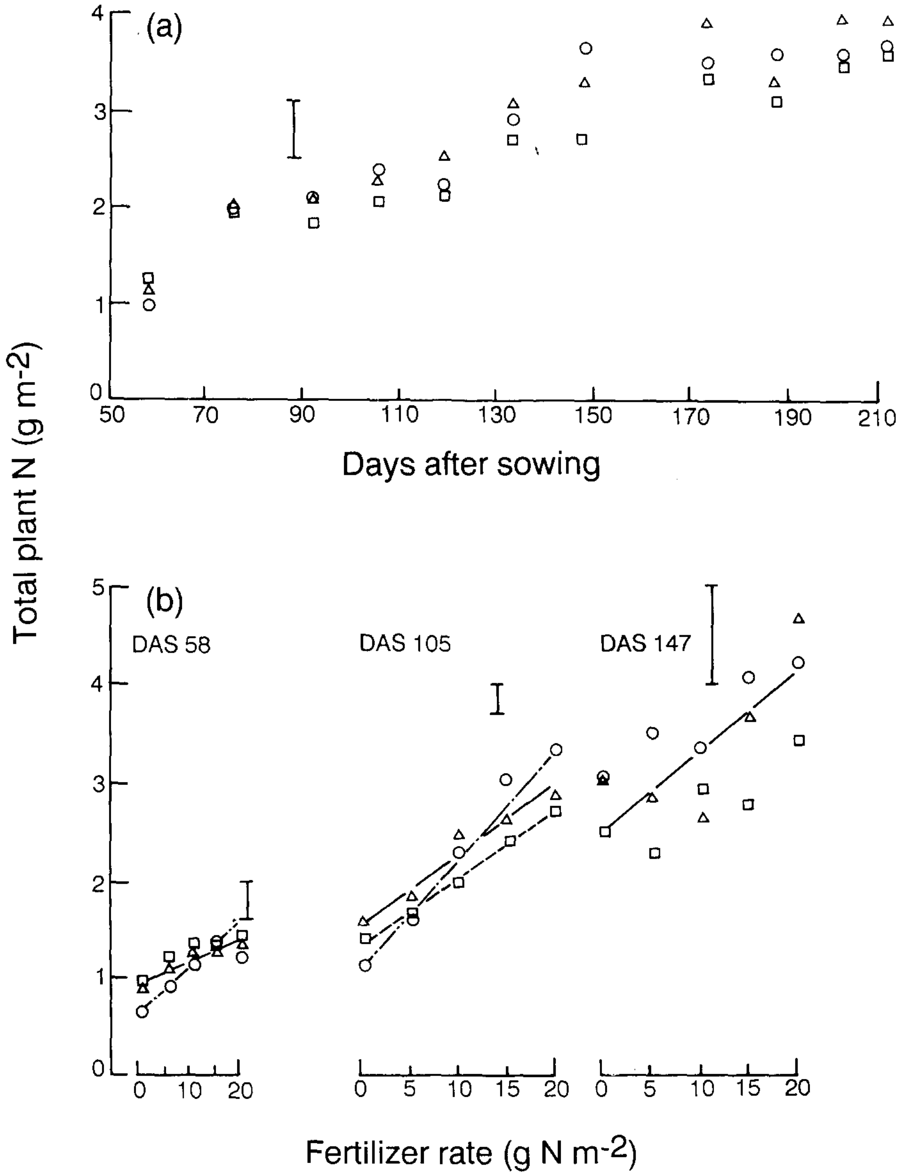

Fig. 6. Effect of: a), inhibitor rate on the mean total N accumulation by wheat tops with time; and b), inhibitor and fertilizer rate on total N accumulation at 58, 105 and 147 DAS. Treatments I_0 (□), I_2 (△), and I_4 (○). Vertical bars represent L.S.D.'s ($P = 0.05$) appropriate to comparisons with: a) time by inhibitor; and b), inhibitor by nitrogen at the selected time.

Mean spike density was $550\,m^{-2}$, with no significant effect of inhibitor. Nitrogen significantly ($P < 0.001$) increased spike density; mean values were 440, 558, 590, 603 and 560 for treatments N_0, N_5, N_{10}, N_{15} and N_{20}, respectively. The higher values associated with increased N addition are comparable to values reported from crops that yield up to $7\,t\,ha^{-1}$ in the same region [25]. The fertilizer treatments appeared to be sufficient to maximise spike number. Kernels per spikelet in these crops varied from 12.5 to 16.8 and are comparable with the findings of Whitfield et al. [25]. However, individual kernel weight was low (average for all treatments was 24.8 mg) and typical of crops water stressed during the growth of the grain [4, 5, 25]. Evidently, the wet winter conditions (see Fig. 1) restricted crop root growth as evidenced by the lack of water extraction below 0.3 m at 155 DAS and below 0.5 m at harvest (Fig. 3). Furthermore,

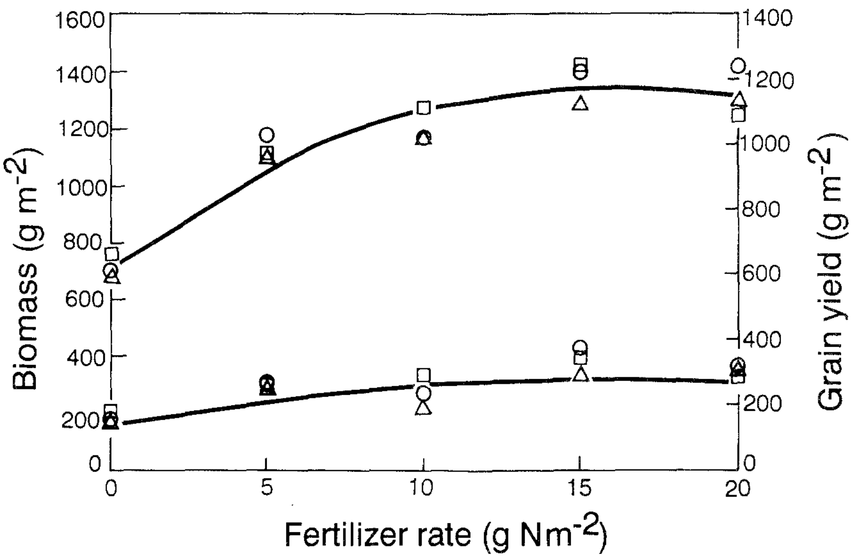

Fig. 7. Effect of inhibitor and N fertilizer on biomass (open symbols) and grain yield (solid symbols). Treatments I_0 (□), I_2 (△), and I_4 (○). Vertical bars represent L.S.D.'s ($P = 0.05$) for comparison between nitrogen by inhibitor.

the soil water content was close to the upper limit of storage at harvest [23].

Grain yields were low in comparison with the biomass production and the average harvest index of the crops was 0.22. Although the crop was irrigated, the limited root development below 0.3 m evidently meant that there was less than 40 mm of plant available water for the crop. The magnitude of the grain yield loss due to water stress and small grain size can be illustrated by recalculating the grain yields using a kernel weight of 44 mg reported by Whitfield et al. [25] for crops which have comparable spike density and kernel per spike. Estimated grain yields are 280, 460, 480, 590 and 540 g m^{-2} for treatments N_0, N_5, N_{10}, N_{15} and N_{20}, respectively.

Grain N concentration had a significant linear ($P < 0.001$) response to urea application (Fig. 8). However, the response to the inhibitor was best described by a quadratic function ($P < 0.001$). Mean grain N concentration of treatments I_0, I_2, and I_4 were 23, 26 and 26 g N [kg oven dry grain]$^{-1}$, respectively.

Effect of inhibitor on recovery of applied N

Recovery of fertilizer N in the wheat plants was significantly increased ($P < 0.05$) by the addition of inhibitor at rates in excess of 2 g m^{-2} (Table 1). The effect of the inhibitor was not expressed when the plants were young and levels of available N in the soil were high. At 101 DAS the effect of the inhibitor treatments I_4 and I_8 was marked and the effect persisted until harvest. There was no further uptake of N by, or loss of N from, the plants in the period 101 DAS to 215 DAS.

The effect of the inhibitor treatments on recovery of applied N by the soil component was apparent by 33 DAS. By that time significantly more N remained in the soil in the three treatments which had received inhibitor than in the treatment without inhibitor. There was also a significant increase in the recovery of applied N with increased application of inhibitor (Table 1). In the control treatment (I_0) 50% of the applied N had been lost from the plant-soil system by 215 DAS, and most of this loss occurred in the first 75 days after application. The loss was reduced significantly by all of the inhibitor treatments until 178 DAS, and by the I_4 and I_8 treatments until harvest (Table 1). The I_8 treatment completely prevented N loss from the plant-soil system for at least 75 days, and significantly increased recovery of N in the plant-soil system at harvest.

Previous work had shown that ammonia volatilization and leaching were not important path-

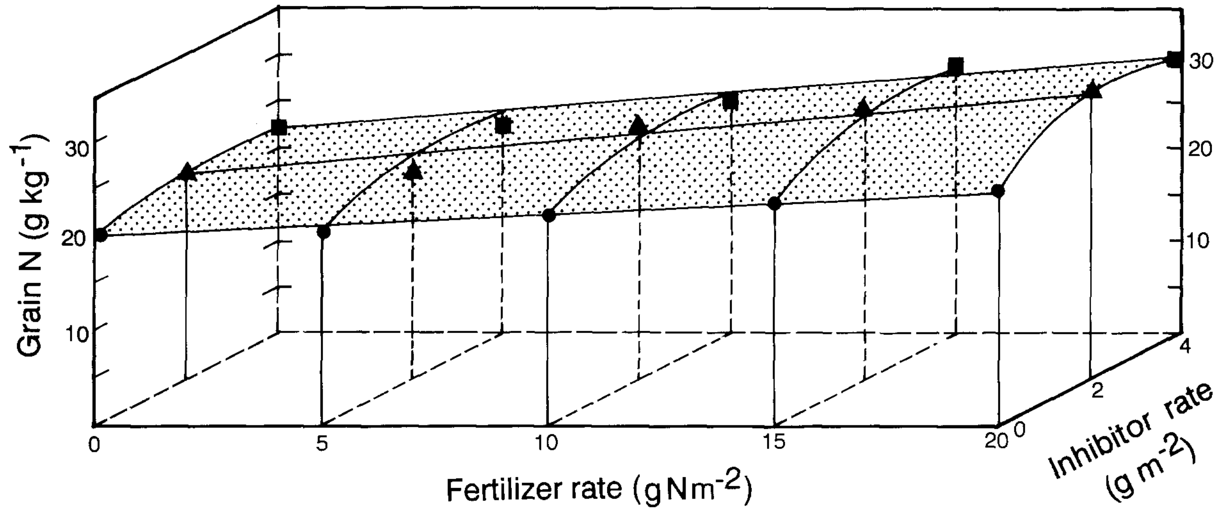

Fig. 8. Effect of inhibitor and N fertilizer on total N in wheat grain at harvest.

ways for N loss from red-brown earths when urea was applied in the irrigation water or broadcast onto the soil and washed in by sprinkler irrigation [16, 19]. As runoff was controlled it was concluded that losses of N could only have occurred by denitrification. In the current experiment, where the fertilizer was placed 0.15 m into the acid loam, there was even less chance of nitrogen being lost by ammonia volatilization. As in previous studies [16, 19] there was no evidence for leaching loss and runoff was controlled. Consequently it was concluded that all losses were due to denitrification, and that the inhibitor treatments reduced NO_3^- production and therefore denitrification.

Even though the soil was saturated for much of the growing season (see above) it is surprising that losses of N from the inhibitor treatments continued to occur after 75 days because by that time inorganic N in the soil had been reduced to low levels, and stayed at the same level until harvest. Consequently N loss by denitrification would be expected to be small in the period 75 DAS to 215 DAS. However, a continual low rate of N loss coupled with replenishment of the inorganic pool by mineralization of organic N during wetting and drying cycles is a possible explanation of the results.

Table 1. Effect of inhibitor on recovery of applied N (%) in wheat plants and soil

Time DAS	Plant				Soil				Total			
	I_0	I_2	I_4	I_8	I_0	I_2	I_4	I_8	I_0	I_2	I_4	I_8
19	1.2	1.2	0.8	1.2	92	98	99	100	93	99	101	101
33	9	10	9	7	75	90	92	98	85	100	101	105
53	12	12	12	10	–[a]	–	–	–	–	–	–	–
75	18	21	23	23	37	53	72	82	55	75	95	105
101	24	22	33	35	34	53	53	52	58	75	87	86
149	25	24	30	36	34	48	47	49	59	72	78	85
178	23	28	28	35	28	38	43	50	52	65	71	85
215	25	21	34	37	25	30	34	36	50	51	68	73
L.S.D.												
T	3.7**				4.5**				4.6**			
I	1.5**				4.2**				4.6**			
T*I	7.0**				9.6**				9.6**			

[a] – not determined.
** $P < 0.01$.

Conclusions

The results show that wax coated calcium carbide effectively inhibited NO_3^- production and denitrification for long periods. The inhibitor also increased N assimilation by the wheat plants and significantly increased the N concentration in the grain. This inhibitor appears to be an effective tool for improving N use efficiency in wheat.

Acknowledgments

This work was supported by a grant from the Wheat Research Council. The authors thank Ms F. Robertson, Ms M. Ferguson and J.W.B. Smith for their excellent technical assistance in the course of this study.

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