IFA International Workshop on Enhanced-Efficiency Fertilizers

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UREASE INHIBITORS

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"Urease inhibitors"

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Urea is the predominant source of fertiliser N used in agriculture throughout the world, accounting for 50% of total world N consumption. Its market share is increasing because of its high N content (46%) and relatively low production cost. However, the efficiency of urea is decreased by losses of N as ammonia gas after the urea is hydrolysed at the soil surface by reaction with the enzyme urease. This loss can account for up to 47% of the urea-N applied under field conditions and is worst when urea is surface-applied in warm conditions to wet soil. As well as having financial implications, ammonia lost to the atmosphere from applied urea will subsequently be deposited to land or water causing eutrophication and acidification, particularly in sensitive ecosystems. Loss of ammonia is one of the reasons why ammonium nitrate (AN) or calcium ammonium nitrate (CAN) has been the main form of N used in the EU for many years. Ammonia loss from AN or CAN is low, but it is more expensive per kg N than urea and can be susceptible to nitrate losses in wet conditions.

The efficiency of urea can be altered by the use of slow release systems, chemical additives (e.g. acidifying agents, soluble salts), by altering granule size or soil incorporation (Watson, 2000). Most of these strategies have proved useful techniques to reduce ammonia emissions from urea fertiliser under specific circumstances, but their adoption into more general farming practice is unlikely to be practical, at present.

One of the most promising ways to improve the efficiency of urea is to use a urease inhibitor. This slows the conversion of urea to NH_4^+, and hence reduces the concentration of NH_4^+ present in the soil solution and the potential for NH_3 volatilisation and seedling damage. Slowing the hydrolysis of urea allows more time for the urea to diffuse away from the application site or for rain or irrigation to dilute urea and NH_4^+ concentration at the soil surface and increase its dispersion in the soil.

Urease inhibitors are expected to be most beneficial on soils when (i) loss of NH_3 from urea fertiliser is high (ii) incorporation of urea is difficult (iii) there is little opportunity for the urea to move into the soil with infiltrating water and (iv) the soil surface has a high urease activity due to lack of cultivation or the accumulation of organic matter.

Thousands of chemicals have been evaluated as soil urease inhibitors and they can be classified according to their structures and how they are thought to interact with urease (Watson, 2000). They can interact with either the enzyme active site or a key functional group elsewhere in the molecule, which may change the conformation of the active sites and preclude urea hydrolysis. Four main classes of inhibitor have been proposed: (i) reagents which interact with the sulphydryl groups (sulphydryl reagents), (ii) hydroxamates, (iii) agricultural crop protection chemicals, and (iv) structural analogues of urea and related compounds. However, few meet the requirements of being effective at low concentrations, non toxic, stable, inexpensive and compatible with urea.

Considerable interest has recently developed around the organophosphorus inhibitors, particularly the phosphorodiamidates, the phosphorotriamides and the thiophosphorotriamides . These compounds are structural analogues of urea and are some of the most effective inhibitors of soil urease activity blocking the active site of the enzyme. The most promising and widely tested soil urease inhibitor at present is N – (n-butyl) thiophosphoric triamide (nBTPT), where the urease inhibitory activity is associated with the formation of its oxygen analogue. NBTPT is highly effective at low concentrations reducing ammonia loss and increasing the yield and N uptake of a range of crops, compared to untreated urea. It can also prevent the adverse effect of ammonia and nitrite toxicity on seed germination and seedling growth following rapid urea hydrolysis, and it has the potential to reduce ammonia loss from livestock waste.

Field trials

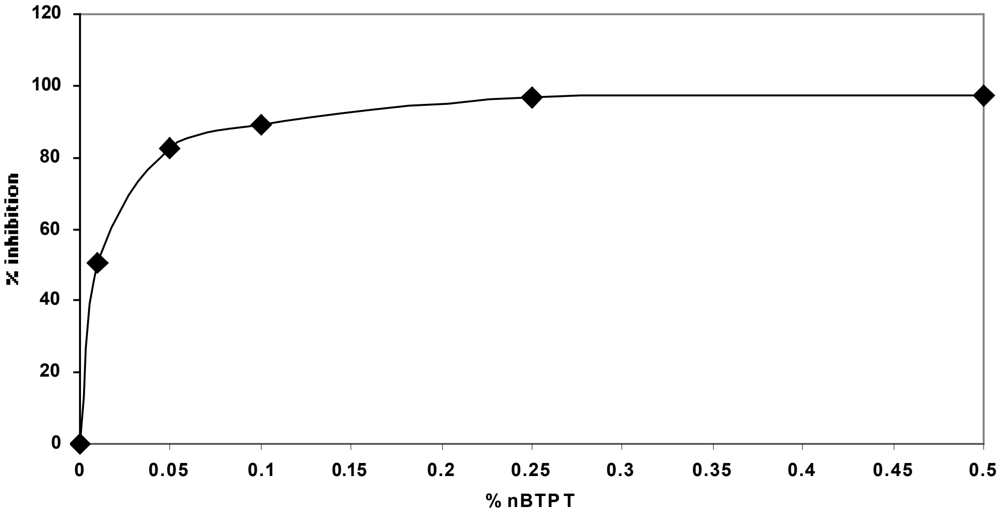
Numerous field trials involving corn and solid urea plus nBTPT have been conducted in the US for 14 years in over 19 states. These have involved 22 universities and 7 private research companies. The average increase in yield to nBTPT addition in 316 replicated 'nitrogen responsive' sites was 14.2 bushels per acre (0.89 t/ha). In contrast to solid urea, urea ammonium nitrate (UAN) solutions treated with nBTPT resulted in an increase in corn yields of 9.0 bushels per acre (0.56 t/ha), when averaged for 119 replicated sites (Trenkel, 1997). Results from numerous field trials undertaken in various states in the USA are summarized on the web site *www.agrotain.com*. The range of crops studied include corn, wheat, barley, rice, cotton, grain sorghum, ryegrass, brome grass and sugarcane. Urea, ammonium nitrate and UAN solutions and nBTPT (0.14% w/w) treated urea have been compared. The results show that generally nBTPT significantly increases the yield from urea.

In Canada, Grant and Bailey (1999) showed the effect of seed-placed urea and nBTPT on emergence and grain yield of barley. Dry-matter yield at heading was frequently not increased by use of nBTPT but grain yield was generally higher when nBTPT was used than in its absence.

Few field trials have been undertaken in Western Europe with urease inhibitors. NBTPT was found to reduce NH_3 volatilisation in field experiments with maize in Italy, and with sugarbeet in Turkey (Trenkel 1997). In Northern Ireland nBTPT (at 0.5% w/w) lowered cumulative NH_3 loss from urea when surface applied to perennial ryegrass in mid summer and delayed by approximately 5 days the time at which maximum NH_3 loss (Tmax) occurred (Watson, *et al*., 1990). Delaying Tmax increases the chance of rain falling to move the urea below the soil surface and hence lower NH_3 volatilisation. The recovery of N by difference and DM yield of the amended urea treatment were increased by 20% and 8.8% respectively compared to urea alone, making the yield performance of urea plus nBTPT comparable to that of calcium ammonium nitrate.

Further field trials evaluated a range of concentrations of nBTPT (0.01, 0.05, 0.1, 0.25 and 0.5% nBTPT w/w) to determine the optimum incorporation rate for temperate grassland under a range of environmental conditions (Watson *et al.* 1994b). Increasing the inhibitor concentration lowered NH_3 volatilisation according to a law of diminishing returns (Fig. 1). The inhibitor was very effective at low concentrations, resulting in approximately 50% inhibition at a concentration of 0.01%. There was little benefit in using concentrations above 0.1% nBTPT on a range of grassland soils (Watson *et al.* 1994). However, as nBTPT is less effective at high temperatures (Carmona *et al.* 1990), higher concentrations may be required under tropical conditions.

Figure 1: Effect of different levels of nBTPT on the % inhibition of NH_3-N volatilisation



A soil incubation study, using a wide range of soil types, indicated that the effectiveness of nBTPT in lowering NH_3 volatilization was greatest in soils with a high pH and low buffering capacity (Watson *et al.* 1994). As these were the soil conditions leading to high NH_3 loss from unamended urea, nBTPT clearly has considerable potential to improve the efficiency of urea for temperate grassland. In addition, there is no evidence of any long-term adverse effect on grass production with repeated applications of nBTPT-amended urea over a 3 year period and no indication that its efficacy to reduce NH_3 loss from urea-treated swards declined when used repeatedly on the same soil (Watson *et al.* 1998).

Trials with barley under conventional and zero tillage have shown that nBTPT had no effect on N mineralisation or on the size and activity of the soil microbial biomass (Banerjee *et al.* 1999). In addition nBTPT does not inhibit nitrification or denitrification (Bremner *et al.* 1986).

Short-term physiological implications

Solution culture studies have shown that urea can be taken up by plant roots as the intact molecule (Harper 1984). In soil, urea is normally rapidly hydrolysed to NH_4^+ -N, so plants would rarely take up urea as the intact molecule. However, if urea hydrolysis in soil is delayed by a urease inhibitor then urea can be taken up by ryegrass as the intact molecule, albeit at a significantly slower initial rate of uptake than NH_4^+ -N. Transient leaf tip scorch can occur approximately 7-15 days after application of urea amended with a urease inhibitor (Krogmeier *et al.* 1989; Watson and Miller 1996). Leaf tip scorch was greatest with high concentrations of nBTPT (0.5%) and high urea – N application rates. However, new developing leaves showed no visual sign of tip necrosis. It is not clear whether the phytotoxicity is due to urea *per se* or as a result of pH fluctuations within the plant tissue following its hydrolysis by shoot urease. Watson and Miller (1996) showed that nBTPT – amended urea markedly reduced shoot urease activity of ryegrass for the first few days after application compared to unamended urea.

The higher the nBTPT concentration the longer the time required for shoot activity to return to that in the unamended treatment. At the highest inhibitor concentration of 0.5% shoot urease activity had returned to that of unamended urea by 10 days. Root urease activity was unaffected by nBTPT in the presence of urea but was affected by nBTPT in the absence of urea. Watson and Miller (1996) studied the composition of amino-acids in roots and shoots of ryegrass and suggested that urea-N within the plant was not metabolised in the same way as N taken up in the NH_4^+-N form.

Although nBTPT – amended urea affected plant urease activity and caused some leaf-tip scorch the effects were transient and short-lived. The previously reported benefits of nBTPT in reducing NH_3 volatilisation of urea and increasing yield would appear to far outweigh any of the observed short-term detrimental effects.

nBTPT and seed-placed urea

Urea has an adverse effect on seed germination and seedling growth in soil (Tomlinson 1970) because of the accumulation of high NH_4^+-N and NO_2^- -N concentrations in close proximity to the seed following rapid hydrolysis of urea by soil urease. NBTPT has been shown to reduce seedling damage from seed-placed urea (Wang *et al.*, 1995). Field trials in Canada showed that nBTPT significantly reduced barley seedling damage and improved seedling emergence at N rates where damage from seed-placed urea occurred (Grant and Bailey 1999).

Control of N loss from livestock waste

Current waste management systems for cattle feedlots and swine facilities result in N losses of approximately 75%. Most of this loss occurs through NH_3 volatilisation following the rapid hydrolysis of urinary N (urea). This contributes to odour, environmental problems and loss of a valuable fertiliser resource. N-(n-butyl) thiophosphoric triamide (nBTPT) and other urease inhibitors have been shown to delay the hydrolysis of urea and reduce NH_3 emissions from livestock facilities, increasing the fertiliser value of the livestock wastes by improving the N:P ratio for plant growth (Varel *et al.* 1999). The most effective method of preventing urea hydrolysis was to spray the surface of the manure with the urease inhibitors, once per week. Further work is required to determine whether urease inhibitors applied to livestock manures are cost effective. The di- and tri-amide urease inhibitors are not toxic to animals, do not have antibacterial activity and are not known to present any environmental problems when used at concentrations which effectively inhibit urease activity. These are important considerations if such inhibitors are to be used in or around animal facilities.

In addition, nBTPT has been used to reduce the rate of NH_3-N release from dietary urea in *in vitro* digestion experiments and therefore has the potential to improve non-protein N utilisation in ruminants (Ludden *et al.* 2000).

A commercially available urease inhibitor (Agrotain)

Neem coated urea and nBTPT are the only urease inhibitors that are currently commercially available. Use of neem coated urea is confined to India, where it has been shown to improve N use efficiency and lower losses. Neem is the press cake from the production of neem oil (from the Indian neem tree, *Azadirachta indica*). However, the performance of neem coated urea is not always reliable (Trenkel 1997).

NBTPT was initially developed by Freeport McMoRan and licensed exclusively to IMC-Agrico. It was first introduced for the American corn market in spring 1996, under the trade name of Agrotain. A new branch of the company was launched in July 2000 called 'Agrotain International' and they now have licensed Agrotain or are selling Agrotain-containing products in over 50 countries. The Department of Agriculture and Food in Dublin has given approval for Agrotain - treated urea to be used in Ireland (S.I. No. 205 of 2004). Summit Quinphos have been selling a product called Sustain® in New Zealand for several years. Sustain green is granular urea, spray impregnated with Agrotain at 1 litre per tonne of urea. Sustain yellow is similar but elemental sulphur is bonded to Agrotain treated urea to give a product containing 4% S. In September 2003 Hydro-Agri (renamed Yara in March 2004) secured exclusive rights for agricultural use for Agrotain in Europe by a licence agreement with Agrotain International LLC, USA. Yara are currently seeking registration of the product as an EC fertiliser under the trademark 'Amiplus'. In March 2005, Agrotain International LLC announced its intention to licence the Agrotain technology to Incitec Pivot Ltd for urea and UAN incorporation at the manufacturing level, for agricultural markets in Australia.

Agrotain is formulated as a green clear liquid containing 20 - 25% of the active ingredient nBTPT. The nBTPT is in a mixed solvent consisting of 10% by weight of N methyl-pyrrolidone with the balance consisting of a non-hazardous solvent and inert ingredients (IMC-Agrico 1997). This can be used to impregnate urea granules, be added to the urea melt during manufacture, or be added to urea-ammonium nitrate solutions prior to surface spreading in the field. Agrotain International recommend a rate of 0.11 to 0.14% for spray impregnation (1.1 to 1.4kg of active ingredient per metric ton of urea). This would require a concentrate loading of approximately 3.91 to 5.21 litres/t of urea. However, lower rates of application have been shown to be highly effective (Watson, 2000). The shelf life of Agrotain coated urea is dependant on storage conditions. A new stabilisation technique (patent held by Agrotain International) has shown that Agrotain incorporated within the urea granule is stable for several years (Semple 2003, personal communication). In dry bulk blends the urea should be impregnated prior to the introduction of other fertiliser materials. For urea ammonium nitrate (UAN) solutions, Agrotain International recommend a rate of 2.0 to 2.7 litres per metric ton for a 30% nitrogen solution. This amended UAN solution should be applied to the field soon after mixing, as nBTPT gradually decomposes in the presence of water.

Agrotain has successfully passed extensive toxicological and environmental tests and degrades into fertiliser elements N, P and S after approximately 10-14 days. It is compatible with most agricultural chemicals (IMC-Agrico 1997).

Agrotain has been used on a wide variety of crops. It is primarily recommended for pre-plant surface application of urea and urea-containing fertilisers but can be used as pre-emergence, side dressing, top-dressing or other post-planting applications. Agrotain is also showing potential as a safener when urea is placed in close proximity to small grain cereals and canola (Sutton 2000 Personal communication) and has been shown to significantly reduce N leaching compared to unamended urea in a fine sandy soil (Prakash *et al.*, 1999). A liquid combination of Agrotain and a nitrification inhibitor is marketed under the label of 'SuperN' as an additive to livestock waste or UAN to prevent N losses. Other commercially available granular products which are registered trademarks of IMC Phosphates Company, licensed exclusively to Agrotain International LLC are SuperU, UFLEXX and UMAXX which contain varying proportions of nBTPT and the nitrification inhibitor dicyandiamide (DCD). These products are for the amenity and horticultural market and are regarded as slow release products. The distributors for these products in the UK are Headland Amenity and Vitex (Semple 2003, personal communication).

Economics

The potential economic benefit of amending urea with a urease inhibitor will depend on the price differential between urea and AN or CAN, the additional cost of amendment (formulation costs and concentration of inhibitor), the amount of N saved from NH_3 loss and the value of the additional crop yield.

In comparing the economics of amended urea and CAN the lime requirement of the two fertilisers needs to be considered. Soil acidity is influenced by ammoniacal fertiliser sources which produce H^+ ions during nitrification. Generally 1.8 t $CaCO_3$ is required to neutralise the acidity of 1 t N as urea. Acidification caused by CAN is partly compensated by its $CaCO_3$ content (~21%), so that 1 t lime is required to neutralise the acidity of 1 t N as CAN. Use of amended urea instead of CAN would require an extra 0.8 t lime per t N, which would have a cost implication. The lime requirement of ammonium nitrate and urea are the same.

Future considerations

Urea fertiliser has advantages over other N fertilisers in being the most concentrated N source available and cheap to manufacture. It has the disadvantage of losing N by NH_3 volatilisation and adversely affecting seed germination and seedling growth in soil. Urease inhibitors effectively overcome these disadvantages. Maximum benefits of urease inhibitors will occur when crop yield potential is high, soil N levels are low and soil and environmental conditions promote extensive volatilisation losses. The development and introduction to the market of new, effective, low price and non toxic urease inhibitors is a time-consuming process requiring years of data collection for registration purposes. The urease inhibitor Agrotain (nBTPT) leads the field in this respect, having been introduced onto the US market in spring 1996.

The expanding acceptance and commercialisation of urease inhibitors world-wide will be dependent on economic, agronomic and environmental considerations. Amending urea with a urease inhibitor has the potential to be cost effective and have environmental advantages over the more expensive AN/CAN. Losses of nitrate by leaching and denitrification should be lower with urea amended with a urease inhibitor however, this requires further quantification under field conditions. Care is required in the future use of urease inhibitors to ensure that they do not increase the risk of urea loss to surface waters, which would increase ammonium-N concentrations above the low EC guideline values for freshwaters.

Although numerous studies have shown the efficacy of urease inhibitors to lower ammonia volatilisation from surface-applied urea, the N saved is not always translated into a yield response. Plant nutritional / physiological aspects may be involved and require further investigation.

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