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THE USE OF UREASE INHIBITOR FERTILIZERS (AGROTAIN) AND THEIR EFFECT ON CEREAL CROPS AND COTTON YIELD

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ABSTRACT

Many commercial compounds exist that promise the increasing efficiency of urea fertilizers by inhibiting urease activity in soils. Such a compound gaining in commercial importance in the last decades is N-(n-butyl) thiophosphoric triamide, broadly known with its registered trade name of “Agrotain”. In this paper, the effect of nitrogen fertilizer dressings using Agrotain versus conventional (urea) fertilizers was studied under field conditions. In particular, the effect of three different nitrogen dressings using conventional N-fertilizers and Agrotain was investigated on the growth and final yield of (rainfed) durum wheat, and (irrigated) maize and cotton, grown on a fertile clay loamy soil in Velestino (Thessaly plain) area in central Greece in the year 2015. It was demonstrated that all three crops fertilized Agrotain obtained greater chlorophyll contents and reached significantly higher biomass and grain yields comparing to the crops receiving traditional nitrogen fertilization, obviously due to the more effective nitrogen release and uptake by the crops.Therefore, application of urease inhibitor fertilizers, such as Agrotain, might reduce nitrogen application dressings, reduce N-losses and nitrification, and their introduction to existing crop rotations is highly advisable.

Keywords: *urea inhibitors, yield, chlorophyll, wheat, cotton, maize.*

INTRODUCTION

Nitrogen is an essential plant nutrient and key to maintaining higher yield production and worldwide economic viability of agricultural systems. Farmers apply different N fertilizers such as urea, ammonium nitrate, ammonium sulphate and potassium nitrate to increase yields. However, this increase in N use, with N-response efficiency reported to be between 33 and 50%, is contributing to higher worldwide N losses via NH_3 volatilization and NO_3^-leaching that impact air and water quality (Raun and Johnson, 1999; Howarth et al., 2002; Nosengo, 2003).
Nitrogen is a major essential element for plant growth and is limited in agricultural soils (Kawakami et al., 2013). For its high N content (460 g N kg^{-1}), urea has been used as the primary solid N-fertilizer in agricultural crop production (Soares et al., 2012; Glibert et al., 2006). However, the increase of pH and surface soil NH_4^+

concentrations resulting from urea hydrolysis can exacerbate NH_3 emission. This can cause low Nitrogen Use Efficiency, especially in alkaline soil or soil with low sorption capacity, which limits the use of urea fertilizer in Europe (Sommer et al., 2004). Nitrogen loss by NH_3 emission not only brings about economic loss to farmers, but also detrimental effects to ecosystems and human health (Bremner, 1995).

There is a need to improve the efficiency of urea-based fertilizers through new technologies and management approaches. One of the most promising approaches is to apply urea in combination with the urease inhibitor (N-(n-butyl) thiophosphoric triamide, nBTPT or NBPT) at low concentrations ranging from 0.01 to 0.5% (NBPT, w/w) (Watson and Miller, 1996; Rawluk et al., 2001; Sanz-Cobena et al., 2008). Urease inhibitor (NBPT) is commercially available under the trade name of Agrotain. Agrotain refers to a liquid product containing 25% NBPT as the active ingredient. Granular urea applications with NBPT have been reported by a number of researchers to be effective in delaying urea hydrolysis as well as increasing productivity under a range of cropping and pasture systems (Chen et al., 2008; Martin et al., 2008).

Urease inhibitor (NBPT) is commercially available under the trade name of Agrotain. Urease inhibitors inhibit the enzyme urease, decrease the urease activity and block the hydrolysis of urea to NH_3 (Varel, 1997). Urea can damage the seedlings after it hydrolyses by the enzyme urease, where the produced ammonia (NH_3) and ammonium (NH4+) can cause ammonia toxicity and osmotic damage (Bremner, 1995). Urea toxicity can be reduced by applying urease inhibitor to the fertilizer granule (Grant and Bailey, 1999; Malhi et al., 2003, Karamanos et al., 2004).

This study was conducted in the main agricultural plain (Thessaly) to evaluate the effect of new types of fertilizers containing urease inhibitor on the yield of the main arable crops (durum wheat, cotton and maize) in Greece.

MATERIALS AND METHODS

For the purposes of the study, field experiments were established in East Thessaly (Velestino, Volos). The selected crops were the same with previous year (2014), to assess the impact of a new fertilizer type in their performance: “durum wheat, corn and cotton”, which are the most prevalent arable crops in Greece.

Soil characteristics

Velestino soil is characterized as Calcixerollic Xerochrept according to USDA (1975). It is a clay loam (sand 19-21%; clay 39-41%, silt 38-42%) calcareous (pH = 8.1-8.3) rich in organic matter (2.3-2.7% in soil profile of 40cm).

Cultivation practices

The experimental plots were demarcated by fixed points in previous year 2014, both on the outer perimeter and the sub-plots of each replication (block), as to remain stable the treatments for the study.

Variety and plant density were exactly the same as in 2014 for each crop. Specifically, sowing of durum wheat took place using a modern seeding machine applying 20 kg ha^{-1} variety "*Simeto*" at the end of November 2014. Maize and cotton was sown using a pneumatic precision seeder machine "Gaspardo 520". Maize sowing distances were 75cm between rows and 15cm on each line and took place at the begging of April 2105 using the Pioneer Hi-Bred hybrid "PR32P26". At least the "Flora" variety of Bayer Crop Science (cotton variety) was sown at distances of 95cm between rows and 4,5cm on each line during the first week of May 2105. There was performed pre- and post-emergence herbicide application, as well as manual control of weeds.

Basic fertilization took place one-two days before sowing using a dispenser and then the fertilizer was incorporates using a rotary cultivator. Finally, the irrigation dose for the emergence applied using a sprinkler system and then a drip irrigation system was established.

Experimental design

Durum Wheat

The experimental design of durum wheat was a completely randomized design with 25 fertilization treatments and three replications (blocks). During seeding period, there were applied three different N-levels (60, 120 and 180 kg ha^{-1}), using four different types of fertilizers (2 conventional simple: 20-10-0 and 16-20-0, and 2 with urease inhibitor: 30-15-0 and 20-24-0) as basic fertilization. The rest amount of the N-fertilization (top dressing) was applied using two simple fertilizers (calcium ammonium nitrate 26-0-0 and ammonium nitrate 34.5-0-0), and two with urease inhibitor (40-0-0 and 46 -0-0). Of course in each block there was a blank plot of zero fertilization.

Maize

The same experimental design was used for maize using different amounts and types of fertilizers. Therefore, three levels of N-fertilization was applied for basic fertilization (120, 240 and 360 kg ha^{-1}), using two simple (20-10-0 and 27-7-5M+0.5 Zn) and two with inhibitor urease (30-15-0 and 24-8-8M+0.5 Zn). The top dressing applied using two simple fertilizers: the ammonium nitrate (34.5-0-0) and urea (46-0-0), while in the case of urease inhibitor were used the 40-0-0 and 46-0-0. Of course in each block there was also a blank plot of zero fertilization.

Cotton

In cotton case was used a completely randomized experimental design with 37 treatments, in three replications (blocks). There were applied three levels of N-fertilization (70, 140 and 210 kg ha-1) using six types of fertilizers for basic fertilization. Three of them were simple (20-10-0, 16-20-0 and 15-15-15) and the other three with urease inhibitor (30-15-0 and 20-24-0 and 15-15-15). The top dressing applied on the plots with the simple basic fertilization using the simple fertilizers ammonium nitrate (34.5-0-0) and urea (46-0-0), while for the other plots the fertilizers 40-0-0 and 46-0 -0 with urease inhibitor were applied. Also in cotton case, in each block there was a blank plot of zero fertilization.

Yield

There was used the same methodology with 2014. Specifically, in case of Durum wheat the yield was calculated by harvesting by hand 1m^2 in each plot and after that a threshing of whole plot took place using an experimental harvester machine of the University of Thessaly on 17 June 2015. In case of maize and cotton, 3,75m^2 and 3,8 m^2 were harvested, respectively.

Meteorological data

Meteorological data were recorded in Velestino from the established meteorological station of University of Thessaly.

RESULTS AND DISCUSSION

Meteorological data

In Figure 1 is illustrated the average temperature and precipitation during crop growth (durum wheat, maize, cotton).

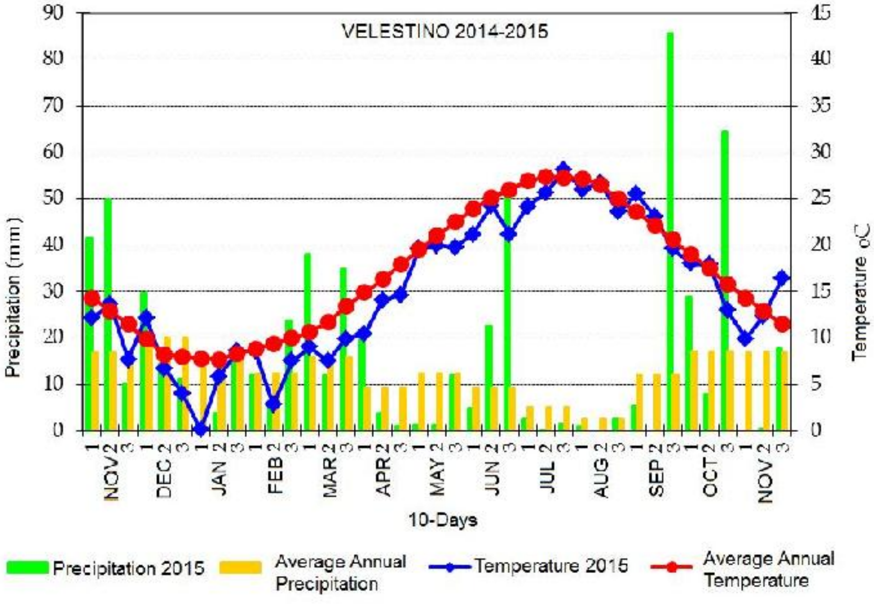

Figure 1.Average air temperature and precipitation at Velestino in 2015

Durum wheat

Normal and increased air temperatures by 2-3 oC for the season were prevailed during durum wheat sowing (late November), which combined with the rainfall in early December resulted in a satisfactory germination without problems in terms of the final population plants and the successful establishment of the crop.

Notable rainfall occurred from late February to early April (114 mm), which favored the growth of wheat in conjunction with the rise of temperature and application of top dressing. Both in April and May unsatisfactory rainfall occurred, so it was considered necessary to add irrigation water in early May, at the end of the crop flowering in both regions.
Heavy rains on June 20 occurred had no effect on durum wheat cultivation, once the harvest had already taken place.

Maize

Couple a days after corn sowing took place; there was recorded satisfactory rainfall (≈ 20 mm) that contributed to a satisfactory germination. Thereafter there was required significant addition of irrigation for the satisfactory crop development, although low precipitation was recorded during summer period. Finally, the rainfall occurred after September 20 postponed crop harvest.

Cotton

Lack of precipitation during sowing time of cotton, necessitating the irrigation application to aid germination. Germination irrigation application resulted in successful establishment and desired plant population. There were not recorded extreme weather events that would cause problems to crop development. High precipitation during September 20 till early October (115 mm) hampered a uniform and satisfactory mature of cotton.

Yield and growth characteristics

Durum wheat

The results have shown differences between fertilization levels for all the examined characteristics, while hysteresis exhibited at low nitrogen level than the other two, which demonstrates once again the importance of fertilization on crop yield.
There was noticed statistically significant difference at the low N-level on chlorophyll and seed yield. On field it was clearly shown that plants fertilized with the lower N-level (60 kg ha^{-1}) were chlorotic with smaller ears.
Chlorophyll content measurements of the plants showed not only a superiority of fertilizers containing urease inhibitor but in some cases also statistically differences. In case of biomass yield, statistically differences were noticed for the different fertilizer combinations in pairs (Table 1). In the case of seed yield which is the main economic product of durum wheat, it appears that fertilizers with urease inhibitor gave higher yields. The amount of 120 and 180 kg ha^{-1} found to have statistically significant superiority compared to the low N-level of 60 kg ha^{-1}. Finally, the 8^{th} fertilizer combination with urease inhibitor reached the higher yield of 5060 kg ha^{-1}.

Table 1. Chlorophyll content, biomass and grain yield of durum wheat under different N-fertilization levels and fertilizer combinations.

Factor		Chlorophyll	Biomass (kg ha^{-1})	Grain (kg ha^{-1})
Nitrogen (kg ha^{-1})	0	16,5	7160	2830
	60	24,9	10940	4290
	120	26,9	11370	4900
	180	29,0	11840	4880
$LSD_{0.05}$		2,62	ns	354,0
Fertilizers	20-10-0 & 26-0-0	22,3	10360	4280
	30-15-0 & 40-0-0 Inhibitor	25,3	11110	4760
	16-20-0 & 26-0-0	26,6	11560	4730
	20-24-0 & 40-0-0 Inhibitor	30,0	12960	5050
	20-10-0 & 34,5-0-0	25,3	10550	4310
	30-15-0 & 46-0-0 Inhibitor	27,8	11440	4610
	16-20-0 & 34,5-0-0	27,7	10850	4730
	20-24-0 & 46-0-0 Inhibitor	30,4	12220	5060
$LSD_{0.05}$		4,28	1670,0	578,0
CV (%)		16,8	15,5	13,0

Therefore, it could be concluded that the N- level of 120 kg ha^{-1} was more profitable, while fertilizers with urease inhibitor produced higher biomass and higher seed yield. Moreover, chlorophyll measurements showed better plant nutrition those which fertilized with urease inhibitor fertilizers. The most efficient combination of fertilizers is the basic with 20-24-0 and the top dressing with 46-0-0.

Maize

The results concerning growth/development and seed yield of maize in 2015 is similar to the results of previous year 2014. Increasing nitrogen fertilization level showed a corresponding increase in chlorophyll content, biomass and seed yield. High hysteresis against all fertilization levels showed the blank plot with zero fertilization, which shows the importance of fertilization on crop yield. It thus becomes clear that the non-use of fertilizers is not an option for reducing the production cost.

The three nitrogen levels showed statistically differences in chlorophyll content with the highest giving darker green plants, which raises expectations for higher productivity and therefore better grain yields (Table 2).

Table 2. Chlorophyll content, biomass and grain yield of maize under different N-fertilization levels and fertilizer combinations.

Factor	Characteristic.	Chlorophyll	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})
Nitrogen (kg ha^{-1})	0	15,2	14560	6300
	120	31,6	26940	12660
	240	44,2	32230	15510
	360	49,1	35100	16460
$LSD_{0.05}$		3,10	2105	999
Fertilizer	20-10-0 & 34,5-0-0	38,0	29730	14260
	30-15-0 & 40-0-0 inhibitor	44,5	31350	15490
	26-7-5 & 34,5-0-0	39,3	30650	13980
	24-8-8 & 40-0-0 inhibitor	41,0	32710	15250
	20-10-0 & 46-0-0	42,5	30860	14480
	30-15-0 & 46-0-0 inhibitor	44,3	31650	14980
	26-7-5 & 46-0-0	40,1	31650	14960
	24-8-8 & 46-0-0 inhibitor	42,3	32770	15620
$LSD_{0.05}$		ns	ns	ns
CV (%)		12,8	11,5	11,6

Although fertilizers with urease inhibitor showed higher chlorophyll levels compared to conventional fertilizers, the differences were not statistically significant (Table 2). Total biomass production showed an upward trend from the lowest to the highest nitrogen level, with statistically significant differences from the lower level to the higher. Urease inhibitor fertilizers compared with common ones in all cases had a numerical superiority.

Finally in case of grain yield, which is the economic crop product, showed that all combinations of urease inhibitor fertilizers outweigh common fertilizers, but there was not found a statistically significant difference. Nevertheless, the mere fact of steadily higher performance of fertilizers with urease inhibitors in all cases, whether comparisons are made in pairs or individually, demonstrated the best provided nourishment versus common fertilizers.

In the case of the of the three N-fertilization levels it was found that the supply of 120 kg ha^{-1} to maize produced 12660 kg ha^{-1} seed, the level of 240 kg ha^{-1} increased the yield to 15510 kg ha^{-1} and final the level of 360 kg ha^{-1} reached the yield of 16460 kg ha^{-1}. Even if the seed yield is increasing by increasing the fertilization level it is cleary shown that the more is applied the smaller is the degree of performance.

Therefore, it could be concluded that the N-fertilization level of 240 kg ha^{-1} using fertilizers with urease inhibitor lead to the same yield with the fertilization of 360 kg ha^{-1} using simple fertilizers, which means probably less Nitrogen losses from leaching and evaporation, and less production costs.

Cotton

Cotton is a less demanding crop in nitrogen, therefore it is not expected any major differences in fertilization. Table 3 shows the chlorophyll content, biomass production and seed yield.

In chlorophyll cases there were found statistical significant differences only between the different N-fertilization levels while among the different combinations of the fertilizers no statistically differences were observed. The higher N-fertilization level the darker green plants were.

In case of biomass production the first N-fertilization level (70 kg ha^{-1}) lags considerably behind the top two levels. The fertilizers using urease inhibitors in all combinations figure slightly over conventional fertilizers.

Cotton seed yield was lower in the first N-level (70 kg ha^{-1}) than the top two N-levels (140, 210 kg ha^{-1}). The blank plot (zero N-fertilization) provides a yield of about 2600 kg ha^{-1}, similar to the results of previous year (2014). Statistical significant differences were found only in case of the different N-fertilization levels (Table 3). Supplying a dose of 70 kg ha^{-1} leads to the seed yield of 3210 kg ha^{-1}, while adding extra 70 kg ha^{-1} the final seed yield increases more or less 460 kg ha^{-1}. Finally at the top N-level the seed yield increased only 80 kg ha^{-1} (Table 3). Among the different fertilizer combinations, compared them in pairs of similar simple and including urease inhibitor fertizers, recorded an increase in yield to the urease inhibitor fertilizers by 6-12%.

Table 3. Chlorophyll content, biomass and seed cotton yield of cotton under different N-fertilization levels and fertilizer combinations at Velestino.

Factor	Characteristic	Chlorophyll	Biomass (kg ha^{-1})	Cotton seed yield (kg ha^{-1})
Nitrogen (kg ha^{-1})	0	35	6300	2600
	70	44,5	8030	3210
	140	48,2	8770	3670
	210	49,5	9200	3750
$LSD_{0.05}$		2,43	610	147
Fertilizer	20-10-0 & 34,5-0-0	46,7	8270	3360
	30-15-0 & 40-0-0 inhibitor	48,7	8820	3620
	16-20-0 & 34,5-0-0	46,2	8210	3450
	20-24-0 & 40-0-0 inhibitor	47,9	8690	3690
	15-15-15 & 34,5-0-0	46,2	8890	3510
	15-15-15 & 40-0-0 inhibitor	51,3	9510	3710
	20-10-0 & 46-0-0	48,0	8430	3360
	30-15-0 & 46-0-0 inhibitor	48,1	8450	3580
	16-20-0 & 46-0-0	44,8	8840	3340
	20-24-0 & 46-0-0 inhibitor	46,7	9060	3650
	15-15-15 & 46-0-0	46,6	8390	3400
	15-15-15 & 46-0-0 inhibitor	47,6	8420	3810
$LSD_{0.05}$		ns	ns	294
CV (%)		10,9	15,0	8,8

CONCLUSION

In the case of Durum Wheat higher values of chlorophyll showed better nutrition of the plants fertilized with a urease inhibitor fertilizer. Fertilizing with a slow-release nitrogen fertilizer gave greater biomass production and ultimately higher yield in durum wheat seed at 35-40 kg ha^{-1}.

In maize case the fertilized plots with fertilizers using urease inhibitor showed higher chlorophyll levels and therefore higher rates of photosynthesis confirming the trend recorded in the previous year (2014). The highest chlorophyll values may have led to higher rates of photosynthesis and thus to increased biomass production perhaps due to smoother and stable nitrogen nutrition. The use of fertilizers with urease inhibitor resulted in increased yield in proportion of 3.5 to 9.1% with an average of 6.4%.

At least in cotton which is a hermit plant and covers a large part of its needs from the soil available nitrogen and therefore the addition of nitrogen fertilizer beyond a limit does not show spectacular results, fertilizers with urease inhibitor showed superiority over conventional in all the studied characteristics. These fertilizers did not confirm the greater superiority in the middle fertilization level also at the second year of experimentation. An average increased yield fluctuated from 6 to 12% with an average of 8% was observed for the fertilizers with urease inhibitor.

Finally as general conclusions it was found that fertilizers with urease inhibitor gave constant voltage supremacy against conventional fertilizers in almost all the studied characteristics for all crops, confirming the results and the trend attributed by experiments of the previous year, in all the studied characteristics. The second N-fertilization level using fertilizers with urease inhibitor gained greater than or equal odds with the high N-fertilization level with simple fertilizers, demonstrating the superiority of these types of fertilizers. The second N-fertilization level was the most effective. Due to the fact that the above results were found through two years of experimentation, safer conclusions expected to arise after the repetition of the experiments in the same place for a third year. In case that the results of the coming year will confirm the previous results then fertilizers with urease inhibitor should be proposed in future fertilization schemes.

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