STUDIES ON THE USE OF UREA AS A FERTILIZER FOR TEA IN CEYLON
2 — UREASE ACTIVITY IN TEA SOILS

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This paper reports studies on soil urease, an enzyme which is essential for the utilization of urea applied to the soil by plants. The urease activity of tea soils from different districts in Ceylon, and its variation with depth, season, and certain cultural practices, such as soil fumigation, fertilizer application and copper fungicide spraying, were investigated.

It was evident that the urease activity of soils varies with location, depth, season and forms of nitrogen fertilizer used. The long-term use of copper fungicide for blister blight control did not significantly alter the soil urease activity, although in laboratory experiments copper inhibited urease activity at levels above 100 ppm. Soil fumigation with methyl bromide destroyed up to 60% of the urease activity, but at two weeks from fumigation recovery has apparently started.

It is concluded that despite the variations and temporary reductions there is always sufficient urease activity present in the soil to hydrolyse the usual amounts of urea applied to the soil. The rate of hydrolysis and hence the time taken for complete hydrolysis at any time would however depend on the actual activity present in the soil at that time.

INTRODUCTION

Although small amounts of urea applied to the soil may be taken up directly by the plant (Webster 1959), the major portion is first converted to inorganic nitrogen (ammonium) before absorption. Early studies (Gibson 1930 and references cited) suggested that the conversion of organic urea to inorganic ammonium carbonate in the soil was a biological process initiated by microorganisms which contained the enzyme urease. But Conrad (1940a and b) showed that the hydrolysis of urea in the soil was to a great extent due to thermolabile catalytic activity, in addition to the microbial action of the microorganisms. It is now clear that this catalytic activity is due to extracellular urease enzyme adsorbed on soil particles (Pinck and Allison 1961). This enzyme is derived from ruptured and moribund cells of microorganisms and plant organs (McGravity and Myers 1967).

The total urea-hydrolytic ability of the soil would therefore be the sum of the urease activity of the ureolytic microorganisms and the extracellular urease activity. Since the latter would depend on the quantity of decomposing plant material in the soil, in addition to the ureolytic microbial population, it can be said that the major factors influencing the urease activity of a soil would be the extent of microbial activity and the organic matter content. Some of the factors that could influence the hydrolysis of urea in the soil are temperature, pH and moisture content.

In this paper the term *urease activity* refers to the extracelluar urease in the soil, measured by using toluene during incubation to inhibit the microbial activity of the soil (Stojanovic 1959; Drobnik 1961) and hence excludes the contribution from this source. *The total urease activity* of the soil was determined in the absence of toluene and includes in addition to the *urease activity* defined above, the activity due to the viable microorganisms.

The present work concerns investigations into the urease activity of soils from the different tea-growing districts of Ceylon, its variability with depth and time of sampling, and the influence of different forms of nitrogen fertilizers on the urease activity. Further, the effects of soil fumigation with methyl bromide and of copper spraying against Blister Blight (leaf disease) on the urease activity of the soil were also investigated. Preliminary observations were also made on the effect of heat sterilization and treatment with toluene on soil urease activity.

MATERIALS AND METHODS

Soil samples from experimental plots or from tea fields were obtained by compositing a large number of soil cores taken with a soil auger at depths ranging from 0-6, 6-12, 12-18 and 18-24 inches. These samples are hereafter referred to as 0-6, 6-12, 12-18 and 18-24 samples respectively. The 0-6 samples were taken after clearing the surface mulch if present. A subsample of the composited soil was placed in a polythene bag, labelled and transported to the laboratory where it was mixed once again and then sieved. The fraction passing through a 1-mm sieve was used for urease and other determinations. The incubation for urease determinations was started on the day of collection, as far as possible. In the case of soils that were transported over a long distance, such as those from the TRI Substations, the incubations were usually started within 24 hours of collection.

Urease activity was determined by the method of Hofman (1963) with slight modifications, as described below.

The sieved undried soil (10 g) was weighed into a 100 ml volumetric flask, 1.5 ml toluene added and the flask stoppered. The contents were mixed well and allowed to stand for 15 minutes. Then 20 ml of citrate buffer of pH 6.7 and 10 ml of a 10% (w/v) urea solution were added, the contents mixed and incubated at 37°C for about 16 hours. For each soil sample, a blank was incubated simultaneously where 10 ml distilled water was used instead of the urea solution. At the end of the incubation, the volume was made up to 100 ml with distilled water, the contents mixed thoroughly and filtered through Whatman No. 1 filter paper. The ammonium contents of the filtrates from the sample and blank were determined by the indophenol blue method (Crowther & Large 1956). The ammonium released by the hydrolysis of urea was obtained by subtracting the ammonium content of the blank from that of the sample. The *urease activity* of the soil is expressed as *urease number*, which gives the quantity of urease contained in 100 g oven-dry soil. For example, urease number 25 corresponds to the quantity of enzyme in 100 g soil which would hydrolyse (or release) 25 mg nitrogen as ammonia from urea, under the conditions of incubation specified.

The procedure for determining *total urease activity* was the same as above except that toluene was omitted from the incubation mixture.

Soil moisture content was determined by drying 10 g sample of soil at 100°C for 24 hours. The pH of the soil was measured in a 1 : 1 soil water suspension using Radiometer pH meter equipped with a glass electrode. The copper content of the soil was determined by the deithyldithiocarbamate method after digestion of the soil with perchloric acid (Jackson 1958).

EXPERIMENTS AND RESULTS

Measurement of urease activity under field conditions

Fresh soil samples (10 g) at their field moisture level were mixed with 1 g urea, sealed in polythene bags and incubated at 37°C. Duplicate sets were removed after one and three days, extracted with citrate buffer (pH 6.7) and the ammonium nitrogen was determined by the indophenol blue method. Parallel determinations of urea hydrolysis were carried out on the same soils by the method described earlier. Toluene was excluded in both determinations. The results of the two determinations are recorded in Table 1.

TABLE 1 — *Hydrolysis of urea to ammonium by soil urease under stimulated field conditions as compared to laboratory conditions*

Incubation period (days)	Mg ammonium-N/100g soil		pH		Soil moisture %
	Field	Laboratory	Field	Laboratory (buffer)	Field
0	—	—	4.05	6.70	25.0
1	110.7	99.8	7.25	6.40	25.0
3	362.2	465.5	9.30	6.60	21.4

The conversion of urea to ammonium under field conditions, as simulated in the laboratory, is comparable to that determined by the routine laboratory procedure up to an incubation period of one day, at 37°C. But when the incubation was continued for three days, the conversion of urea to ammonium under the field conditions is only 78% of that obtained by the laboratory procedure.

Urease activity of toluene-treated and heat sterilized soils

The *total urease activity* of three soil samples sterilized in an autoclave at 15 lb pressure for 15 minutes and that of the untreated soils were determined as described earlier. The *urease activities* of the same soils after toluene treatment were also determined. The results are recorded below.

	Urease numbers		
Soil sample	I	II	III
Total urease activity of untreated soils	50.4	39.8	40.8
Urease activity of the same soils, determined after toluene treatment	30.8	20.5	27.2
Total urease activity of soils after heat sterilization	5.6	1.3	n.d.

The urease activity of the three soils are about 61, 51 and 67% respectively of the *total urease activity*. Sterilization by autoclaving had reduced the *total urease activity* of two soils to about 11 and 3% respectively of the original and this treatment would probably have destroyed the *urease activity* completely.

Urease activity of soils from different districts

The urease activity of soils from the different tea-growing districts was determined by collecting a number of soil samples from the TRI Substations. The range of the urease numbers and the mean values for the different soils are tabulated (Table 2).

A wide range of urease activity was found in the St Joachim and Passara soils as compared to the St Coombs and Hantane soils. Some surface samples (0-6) from the former two stations had urease numbers as high as 100. Comparing the means, it appears that the Passara and Hantane soils have higher urease activity than those of St Joachim at St Coombs soils.

Variation of urease activity of soils with depths

The marked decrease of urease activity with depth is evident in Table 2. This trend was noticed in all the soils investigated. The variation of urease activity in the first three inches of the soil was also investigated. The samples were taken during a dry spell from two areas which had hardly any surface mulch. The urease numbers of the soils were as follows :—

Depth (inch)		0–1	1–2	2–3
Urease number —	Soil 1	20.3	26.7	28.9
	Soil 2	23.0	19.0	20.6

The urease activity of these two soils are low compared with the mean for St Coombs soil (Table 2), probably due to the lack of organic matter in these soils. However, it is apparent that the variation in the urease activity in the top three inches of the soils is minimal compared with the variations in the lower layers. It is also evident that even the dry surface soil (0–1 inch) which is void of mulch has appreciable urease activity.

In a separate experiment the urease activities of the surface mulch, derived from decomposing tea prunings and the soil to a depth of three inches were determined. It was found that the urease activity of surface mulch was very nearly double (45.8) that of the first three inches of soil (24.4). This was expected since an examination of tea leaves earlier had shown high urease activity (Bhavanandan and Fernando 1970).

TABLE 2 — *Urease activity (in urease/numbers) of soils from the TRI Stations*

Location	St Coombs		St Jeachim		Hantane		Passara	
Depth (inch)	Range	Mean	Range	Mean	Range	Mean	Range	Mean
0 — 6	21.8 — 57.5	39.6	24.3 — 103.2	49.7	42.7 — 73.9	60.4	30.3 — 98.4	57.8
6 — 12	20.2 — 28.5	23.7	21.2 — 30.6	25.4	29.5 — 52.4	40.3	26.6 — 72.6	42.1
12 — 18	12.6 — 20.4	14.8	14.0 — 26.4	18.2	22.9 —32.8	26.8	22.4 — 40.6	31.5
18 — 24	11.9 — 16.7	13.9	12.8 — 19.3	14.9	14.5 — 22.8	19.5	21.0 — 40.6	30.9

Effect of different forms of nitrogen on the urease activity of soil

The soil samples for the above study were obtained from Experiment A4 at St Coombs. This experiment was initiated in 1961 to investigate the yield response of tea to three different forms of nitrogen, each at two levels (Tolhurst 1962). The forms of nitrogen in this experiment were originally sulphate of ammonia, urea and a granular formulation, but in 1965 the last treatment was changed to calcium ammonium nitrate due to the unavailability of the granular formulation.

Soil samples (0–6) were taken at weekly intervals from all the plots receiving the three forms of nitrogen at the higher level, *viz.* 300 lb per acre per year, and analysed for urease activity. Since it was our intention to invetsigate also the seasonal effect on urease activity, six samples were collected during the period, August to October 1968, and six during a comparatively dry period, February-March 1969. The range and means of 12 determinations of the urease numbers and of soil pH are recorded in Table 3.

TABLE 3 — *The effect of different forms of nitrogen fertilizer on soil urease activity (in urease numbers) and pH*

(12 determinations)

Type of nitrogen fertilizer	Urease numbers		Soil pH	
	Range	Mean	Range	Mean
Sulphate of ammonia	16.7 — 41.9	24.7	3.65 — 3.95	3.74
Calcium ammonium nitrate	21.7 — 48.2	31.9	3.95 — 4.50	4.18
Urea	32.3 — 59.2	41.0	4.20 — 4.50	4.36
	LSD ($P < 0.001$) — 10.0			
	($P < 0.01$) — 7.5			

The difference in the urease numbers of the soils for the types of nitrogen treatments are highly significant.

Influence of period of sampling on soil urease activity

The urease numbers from the preceding experiment, together with soil moisture, rainfall and sunshine data are tabulated in Table 4.

TABLE 4 — *The effect of period of sampling on soil urease activity (in urease numbers)*

Period and dates of sampling (weekly)	Wet 20.8.68 — 23.9.68		Dry 5.2.69 — 14.3.69	
	Range (6 determinations)	Mean	Range (6 determinations)	Mean
Rainfall per week (in.)	0 — 5.4	3.2	0 — 0.8	0.4
Sunshine per week (hr)	0 — 64.2	22.1	34.8 — 69.4	59.9
Soil moisture (%)	26.9 — 34.3	31.8	14.8 — 28.5	21.1
Urease numbers	16.7 — 46.7	28.4	19.0 — 59.2	36.6*

* Significant at $P < 0.001$

The difference between the urease activity of the soil during the dry period and the wet period is very highly significant. There was no interaction between the types of nitrogen treatment and the periods of sampling. Regression analysis showed that there was a decrease in urease number of 1.73 units for every inch of rain but this relationship was not significant. There was no correlation between sunshine and urease activity.

Influence of copper on the urease activity of soil

Heavy metals such as copper, silver, mercury, cadmium and lead are known to be inhibitors of urease enzyme. Of these elements only copper could be expected to reach problematical levels in the tea soils because of the wide use of copper fungicides for the control of Blister Blight.

The inhibition of urease activity in the soil by copper was investigated in a laboratory experiment by adding known amounts (1-160 mg) of copper as copper sulphate or Perenox (a cuprous oxide fungicide containing 50% copper) to the soil (10 g) before incubation with buffer and urea.

The ammonium released by urea hydrolysis was estimated by the procedure described under methods, since control experiments showed that copper at the levels used did not interfere in the indophenol blue colour formation. The results of the experiments are tabulated in Tables 5 and 6.

TABLE 5 — *Inhibition of soil urease activity (in urease numbers) by the addition of copper sulphate*

Copper added as copper sulphate (mg)	0	10	20	40	80	160
Urease numbers	31.7	21.9	16.9	15.8	13.9	10.0
Inhibition (%)	—	30.9	46.7	50.2	56.2	68.5

TABLE 6 — *Inhibition of soil urease activity (in urease numbers) by the addition of copper fungicide, Perenox*

Copper added as Perenox (mg)	0	1	3	5	7	9	10	20	40	80	160
Urease number	27.2	27.2	25.5	22.1	20.3	18.6	15.3	7.2	4.3	3.4	3.4
Inhibition (%)	—	—	6.1	18.8	25.3	31.5	43.6	73.4	84.2	87.5	87.5

Both forms of copper inhibited the urease activity in the soil considerably at concentrations above 100 ppm. But surprisingly the insoluble form of copper, namely the fungicide Perenox, caused a greater inhibition than the soluble copper sulphate at equivalent amounts. The effect of addition of Perenox and copper sulphate on soil urease activity is illustrated in Figure 1.

In order to investigate whether the continued use of Perenox in tea fields for control of Blister Blight has any effect on the soil urease activity, soil samples from Experiment **P26** at St Coombs were used. This experiment conducted by the Plant Pathology Division is designed to determine the loss of crop caused by Blister Blight on unshaded seedling tea (De Silva 1968), at six different levels of infection. The levels of infection are maintained by spraying 0, ½ 1, 2, 4 or 8 oz of Perenox at weekly intervals. At the time of sampling the experiment had been in progress for about three years. Soil samples (0-6) were collected from all 24 plots, consisting of four replicates of the six treatments, on two occasions with an interval of about a month. The urease numbers for the six treatments, each being the mean of eight values consisting of two determinations on the four replicates, together with the copper content of the soil are recorded in Table 7.

TABLE 7 — *Mean urease numbers and copper contents of the soils from Experiment* **P26**, *St Coombs*

Treatment (oz Perenox/acre/week)	0	½	1	2	4	8
Mean urease numbers	19.5	17.3	17.9	18.1	16.2	14.5
Copper content (ppm) mean of 2 determinations	57.5	85.0	95.0	90.0	70.0	102.5

Although the mean urease number of the soils from the plots receiving no fungicide is higher than those of any from the treated plots, the differences are not statistically significant. The copper content of the soil from the plots receiving no fungicide is the lowest, and that of the soil from the plots receiving the highest dose of fungicide the highest. But there is no consistent trend between the other treatments.

Effect of soil fumigant methyl bromide on urease activity of the soil

Soil samples (0-6) for the above investigation were obtained from four experimental plots before and 2, 7, 14, 21 and 49 days after fumigation. Two plots (200 sq. ft each) were fumigated with methyl bromide (containing 2% chloropicrin) at the rate of 1 lb and two at 4 lb per 100 sq. ft as described by Shanmuganathan (1967). The changes in the urease numbers (mean of two values) of the soil are recorded in Table 8.

TABLE 8 — *Effect of soil fumigation with methyl bromide on the urease activity (in urease numbers) of the soil*

Rate of fumigation (lb/100 sq. ft.)		No. of days from fumigation 0	2	7	14	21	49
1	Urease number (mean)	28.8	17.8	14.7	21.2	18.0	15.8
	%of the value before fumigation	100.0	61.8	51.0	73.6	62.5	55.0
4	Urease number (mean)	25.1	15.9	9.2	9.8	12.7	10.0
	%of the value before fumigation	100.0	63.3	36.6	39.0	50.6	39.8

The decrease in soil urease activity is highest seven days after fumigation at both rates of fumigation. The urease activity had decreased to 51.0% and 36.6% of the original in seven days at the lower and higher rates of fumigation respectively. The recovery of activity was quicker in the soil fumigated at the lower rate as shown by the increase in the urease number after 14 days from 51.0% to 73.6% of the original. In the soil fumigated at the higher rate, the urease activity shows a significant increase only after 21 days, but it is still only 50.6% of the original activity.

DISCUSSION

The importance of soil urease activity in the utilization of urea applied to the soil by the plant is now well established (Bhavanandan 1970a). It is also clear that urease is present in both microorganisms and crop residues. Hence the properties of the soil and climatic conditions would together determine the degree of urease activity and therefore the capacity of the soil to hydrolyse urea.

In this study a well established method (Stojanovic 1959 ; Hofman 1963 ; McGarity and Myers 1967) was chosen for the determination of urease activity and total urease of the soil. This method determines the urease activity of the soil under standard conditions of pH and temperature. Such standard conditions are necessary to get reliable and reproduceable results and to compare the urease activity of different soils. However, when urea is applied in the field, the conditions under which urea is hydrolysed are very different. Firstly, the temperature does not remain constant over the period of hydrolysis ; secondly, the pH of the system changes during the hydrolysis (Bhavanandan 1970b). Finally, even though the moisture in the soil and rain falling after application of urea would dissolve it and bring into contact with the soil, the degree of urea-soil urease contact would not be the same as in the laboratory where a soil-buffer suspension is used. In our experiments field conditions were simulated in the laboratory by adding solid urea to fresh soil obtained from the field. It was evident (Table 1) that after a day's incubation, the amount of urea hydrolysed under the two conditions are comparable ; in fact, about 10% more ammonium was obtained under simulated field conditions. However, after three day's incubation, about 29% more ammonium was detected under laboratory conditions, which is probably due to better mixing of substrate and enzyme. A large increase in pH was noticed under simulated conditions, whereas under laboratory conditions the change in pH was only slight. It was concluded that the laboratory method gives a realistic estimate of the hydrolysis taking place under field conditions, provided that the length of incubation was less than 24 hr and the soil in the field reasonably wet. For all further studies on soil urease the laboratory procedure was therefore used.

Since the extracellular urease activity of the soil would depend on the general ureolytic microbial population, a measure of the former would be sufficient for a comparative study. For this reason, it was decided that for our studies only the *urease activity* need be measured. The contribution of the viable microbial population to the *total urease activity* of the soil was, however, determined in three soil samples. It was found that 49, 39 and 33% of the *total urease* activity was due to the microbial populations and the rest due to extracellular activity. Further, it was also seen that between 89 to 93% of the total urease activity was destroyed when the soils were heat sterilized by autoclaving. Since the extracellular urease activity of the soil is known to be thermolabile (Conrad 1940), it is apparent that in addition to this activity the major portion of the activity due to the viable microorganisms is also destroyed by heat sterlization.

Investigations elsewhere have shown that urease activity of the soil is influenced by soil properties such as colour, texture, reaction, organic matter and moisture (McGravity and Myers 1967 and references cited). Since the overall effect of all these factors would be reflected in the urease activity of the soils, a survey of the soils from the TRI Substations was carried out. The results (Table 2) showed large variations both within and between the stations. It is therefore not possible to classify these soils with any certainty on the basis of the present study. However, an examination of the mean urease activities of the soils shows that Hantane and Passara

soils have higher activity than St Coombs and St Joachim soils. But such conclusions may not be valid since the influence of the time of sampling on urease activity was not known at the time of this investigation was started, and therefore no attempt was made to collect soil samples from the different stations under comparable weather conditions. Subsequent studies on this aspect showed that urease activity varied with climatic conditions and hence time of sampling. In future studies this will be taken into account. Nevertheless, it appears fairly safe to conclude from the results of the survey that the urease activity detected in all the soils examined would be sufficient to readily hydrolyze the amounts of urea added to the soil as fertilizer.

The soil survey studies indicate a clear relationship between soil urease activity and depth. In general, soil urease activity decreased with increasing depth, the lower layers (18-24 in.) having only about 30-40% of the activity recorded in the top six inches. An interesting feature was the high activity observed in the first one inch of soil taken from a dry area, devoid of organic matter. Further, the third inch of soil had higher urease activity than the first and second inches. Thus when urea is broadcast, it is likely that hydrolysis will occur as soon as it is dissolved and brought into contact with the surface soil. Since some loss of nitrogen could occur if hydrolysis began at the surface, it would be preferable if the urea applied is leached down to a few inches below the surface before hydrolysis (Bhavanandan 1969). Moreover, as the urease content in the surface leaf litter could further aggravate volatilization losses, it would be advisable to incorporate by forking the urea applied after pruning and the surface mulch into the soil (Fernando *et al.* 1969).

The results of the experiment on types of nitrogen showed highly significant differences between the urease activities for the different treatments. Soils from plots receiving urea had the highest urease activity, followed by the soils from plots receiving calcium ammonium nitrate. The urease activity of the sulphate of ammonia plots was the lowest, being about 60% of the activity of urea-treated plots. It is possible that the continued use of urea had increased the urease activity of the soil. This could be a direct effect due to an increase in the ureolytic microbial population of the soil, since when urea is available as a source of nitrogen the tendency would be for microorganisms dependnig on this source for their nitrogen to multiply. But if this was the only reason it would be difficult to explain the higher urease activity of the calcium ammonium nitrate plots as compared to the sulphate of ammonia plots. Therefore, some other factor or factors must also be responsible. Soil properties such as texture, colour, moisture and organic matter content and to a certain extent even nutrient status could all be assumed to be uniform in the experimental area. The only known soil factor that differed between the treatment was soil pH and this could have some influence on urease activity. For example, the soils from the sulphate of ammonia plots were highly acidic (pH 3.74) and would not be congenial for microbial activity and possibly for the stability of the released extracellular enzyme. This might explain the low urease activity of the sulphate of ammonia plots. In this connection, it is interesting to note that the soils from the calcium ammonium nitrate plots which had pH of an intermediate value exhibited urease activity which was higher than that of the sulphate of ammonia plots but less than that of the urea-treated plots. A weekly positive correlation between soil pH and urease activity was also reported by McGarity and Myers (1967).

The results of this work also indicate that urease activity is higher during the dry season, suggesting that continuous wet conditions with very high soil moisture are not suitable for the build-up of soil urease activity. These results which were from an experiment at St Coombs are perhaps typical for the up-country districts, where the dry season is not so pronounced. In areas such as the Uva districts, where the dry season is prolonged, the results may be different.

Investigations into the effects of long-term use of copper sprays have shown that the continued use of copper fungicides at levels now recommended is unlikely to have any significant effect on soil urease activity. Although the urease activity of soils from sprayed plots was always lower than those from soils from unsprayed plots, the differences were not statistically significant. As inhibitory concentrations of copper (>100 ppm) can build-up in the top soil only after the application of about 400 lb of fungicide (50% Cu) and as this would take many years at currently recommended rates, there appears to be no immediate danger of copper spraying interfering with soil urease activity.

Fumigation of the soil with chemicals like methyl bromide is generally considered to destroy a wide variety of microorganisms present in the soil. Fumigation of tea soils before replanting is now recommended for areas infested with root diseases (Shanmuganathan 1967) and, in some instances, as an alternative to rehabilitation with Guatemala grass for areas infested with parasitic nematodes (Sivapalan 1969). It is also recommended as a routine treatment for the control of parasitic nematodes in nursery soil (Kerr and Vytilingam 1967). Results of experiments reported here have revealed that soil fumigation with methyl bromide causes a marked decrease in urease activity. It is first evident two days after fumigation, probably due to immediate inactivation of the extracellular urease. The decrease is at its maximum seven days after treatment, when it is likely that there is no replenishment of extracellular urease from viable microbial sources, which would have been destroyed to a large extent. This reduction in activity is, however, only temporary because 14 days after fumigation there is evidence of a renewal of activity, indicating that both microbial population and extracellular enzyme activity were beginning to build-up again. Nevertheless, it appeared that it would take a long time for the soil urease activity of the fumigated soil to reach the original level. In future studies it would be interesting to follow the changes in the *total urease activity* after soil fumigation with methyl bromide. In conclusion, it could be stated that even though fumigation has the immediate effect of depressing soil urease activity, the activity present at the time the first application of fertilizer is made after planting, which is normally some weeks after fumigation, is sufficiently high to hydrolyse the urea applied.

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