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Urea treatment of barley grain. Effect on storage properties and fungal growth

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Abstract. Barley harvested at three different moisture contents (22, 25 and 32 %) was treated with varying levels of urea (1.0, 1.5, 2.0 and 2.5 % on fresh weight basis) and stored aerobically in experimental silos of 400 liters. The chemical composition of barley was analysed before treatment and after 12 months of storage. Fungal contamination of grain samples was analysed.

Application of 1.5 % or more urea effectively preserved the grain for 12 months. At the highest moisture content, 1.0 % of urea did not prevent deterioration during storage. The average recovery of dry matter was 97.1 %, of nitrogen 93.9 %. Ammonia was released from hydrolysis of urea. The degree of hydrolysis increased with increasing moisture content of barley. Raising the levels of urea from 1.0 to 2.5 % significantly increased the quantity but tended to decrease the proportion of urea hydrolysed.

Representatives of 33 genera of fungi and unidentified actinomycetes were present in the grain lots examined. Most fungi were found only occasionally and their incidence declined towards the end of storage. The grain stored at the highest moisture level (32 %) contained more fungi and less actinomycetes than other seed lots. All urea treatments reduced the number of fungi on seeds. Urea concentration of 2 % or more eliminated *Fusarium*- and *Aspergillus*-species on all moisture levels. *Scopulariopsis brevicaulis* (Sacc.) Bain. and other species of *Scopulariopsis* were the most commonly encountered fungi after urea treatment. The total number of *Scopulariopsis* ssp. exceeded 100 % and the amount remained high for 40 weeks of storage until a rapid decline towards the end of storage. Urea treatment also favoured the occurrence of actinomycetes.

Index words: barley, urea, preservation, fungi, *Fusarium, Scopulariopsis*

Introduction

Grain achieves a physiological maturity at a moisture content of 30—40 %. Thereafter, the major change in grain is a loss of moisture (KRALL 1972). In Finland, the moisture content of cereal grains at harvesting time usually exceeds 20 %. The grain must be dried to a moisure content of 15 % or less to prevent nutritional losses and microbial activity.

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Today, more than 90 % of the total grain yield is dried, the rest either being treated with propionic acid or ensiled.

Propionic acid has for long been known to be an effective preservative for high moisture grain. The nutritional value for ruminants and swine appears to be equal to that of dried grain (Jones *et al.* 1974). The preservative effect of propionic and other volatile fatty acids is based on their fungicidal properties.

The efficiency of ammonia as a preservative of high moisture corn has been reported by Bothast *et al.* (1973, 1975) and Britt and Huber (1976). It has eliminated molds and yeasts in initially highly contaminated high moisture corn (Bothast *et al.* 1975). Both liquid and gaseous anhydrous ammonia has been used (Montgomery *et al.* 1980).

Schmidt *et al.* (1978) and Ørskov *et al.* (1979) reported that moist urea-treated grain can be preserved for several months. The preservative effect of urea is also based on ammonia. In moist feeds, microbially produced urease hydrolyzes urea to ammonia and carbon dioxide. Urea offers certain advantages over ammonia; it is easier to handle and nitrogen losses are smaller. The long term preservative effect of urea has been better than that of ammonia (Schmidt *et al.* 1978).

The objective of the present study was to quantify the effect of different levels of urea treatments on some storage properties and the viability of certain fungal propagules on barley grain preserved at three moisture contents.

Materials and methods

The barley lots in the present study were harvested on August 24 and 30 and September 4, 1984. The intension was to harvest at the moisture levels of 22, 27 and 32 % before treatment, but the difference between the two lowest moisture contents was only about 3 %.

At all moisture contents barley was treated with 1.0, 1.5, 2.0 and 2.5 % of urea on fresh weight basis and placed in 400-l experimental glass fibre silos. The silos were covered with plastic and insulated with a 10 cm layer of glass wool to avoid losses of heat produced and left at ambient temperatures for one year. Urea was delivered in a water solution (1:1) to the boot end of a 0.15×5 m grain auger. The grain temperatures were recorded by placing thermocouples in the centre of each silo.

Samples for pH measurements and for analyses of fungal contamination were taken before treatment, 3 days after treatment, then weekly for 10 weeks and subsequently at about monthly intervals. Germination and presence of fungi and actinomycetes were examined by a slightly modified blotter test (de Tempe 1963). Seeds were placed on 14 cm Petri dishes on moist filter paper, 50 seeds per dish. Each dish was moistened with 10 ml of distilled water. The Petri dishes were kept in plastic bags in diffuse day light at room temperature (20 °C). After an incubation period of 14—18 days, the number of germinated seeds was calculated. Each individual seed was studied under a stereo microscope and the fungi and actinomycetes showing growth were identified directly or after isolation on PDA (potato dextrose agar, Difco). The presence of any fungus on individual seed was recorded. No attempts were made to estimate the vigour of growth on individual seeds. A sample of 200 seeds of each moisture content and urea treatment was examined at each sampling time. The total number of seeds examined was 43800.

The chemical composition of barley was determined before the treatment and after unloading the silos. Feed analyses were made according to standard procedures. Total nitrogen (N) and soluble N were determined in fresh samples which were stored frozen. Ammonia N, sugars and volatile fatty acids (VFA) were analysed by the methods described by Huhtanen (1984). Urea was determined as described in the Technical Bulletin 27 of the Ministry of Agriculture, Fisheries and Food (Anon 1973).

In calculation of dry matter (DM) losses, 89 % of VFA was assumed to be lost in oven

drying at 100°C (PORTER *et al.* 1984). Also the weight loss due to hydrolysis of urea to ammonia and carbon dioxide and N lost as ammonia in oven drying were taken into account. Analyses of variance were made according to SNEDECOR and COCHRAN (1967). Differences between urea levels were further partitioned into linear, quadratic and cubic effects. The counts of fungi were studied by the G-test for independence (SOKAL and ROHLF 1969). The results are expressed as percentages of examined seeds.

Results and discussion

Preservation and chemical composition

Urea treated barley kernels were brown in colour. The colour was deeper at high moisture levels. Visible mold growth was apparent only in the silo of the lowest urea level and highest moisture content.

Urea treatment increased the crude protein content from the initial 108 to 190 g/kg DM. The urea level had no effect on ether extract, crude fibre or sugar contents (Table 1). The sugar content decreased from the initial 31.6 to 20.6 g/kg DM. No lactic acid was recovered in the treated barley. Acetic acid fermentation increased with the urea level ($P < 0.01$). Similar low concentrations of acetic acid in urea-treated grain have been observed by SCHMIDT *et al.* (1982). Certain apparently significant effects of moisture contents on chemical composition of grain indicate merely the diversity of grain at the time of preservation caused by different dates of harvest.

pH rose from the initial 6.5 above 8.7 within 3 weeks in the silos which were preserved at a moisture content of 22 or 25 % and within three days in those preserved at the moisture content of 32 %.

The average DM recovery from 12 silos was 97.1 % (SE 0.7 %). It was not affected either by the level of urea or moisture content. Similar DM losses in urea treated grain have

Table 1. Effect of urea level on chemical composition (g/kg DM), pH and dry matter (DM) and nitrogen (N) recoveries.

	Urea level				Moisture content			SEM		Statistical significance		
	1.0	1.5	2.0	2.5	22	25	32	Urea	Moisture	Urea L	Urea Q	Moisture
Dry matter (g/kg)	722	725	726	721	768	743	659	3.6	3.1	NS	NS	***
In dry matter												
Ash	28	28	27	27	30	25	28	0.2	0.2	*	NS	***
Crude protein	142	159	171	190	171	157	169	2.2	2.8	***	NS	*
Ether extract	19.4	19.4	19.3	19.0	21.5	18.8	17.6	0.47	0.41	NS	NS	*
Crude fibre	73	65	71	65	68	71	67	3.5	3.0	NS	NS	NS
NFE[1]	764	768	760	763	749	771	771	3.6	3.1	NS	NS	**
Sugars	21.1	20.4	20.4	20.6	22.7	17.3	21.8	0.9	0.8	NS	NS	**
Acetic acid	0.95	1.16	2.45	2.92	2.08	1.00	2.84	0.29	0.26	**	NS	**
Propionic acid	0.24	0.16	0.14	0.12	0.10	—	0.38	0.08	0.07	NS	NS	**
Butyric acid	—	—	—	+	+	—	—	—	—	—	—	—
Isovaleric acid	—	0.02	0.02	0.01	+	—	0.04	0.01	0.01	NS	NS	NS
pH	8.70	8.85	8.90	8.87	8.81	8.81	8.87	0.03	0.03	*	*	NS
DM recovery (%)	94.3	98.1	98.1	97.9	97.5	97.3	96.3	1.4	1.2	NS	NS	NS
N recovery (%)	94.1	95.6	93.4	92.6	96.1	94.5	91.2	1.1	1.0	NS	NS	NS

[1] NFE = nitrogen free extracts. SEM = standard error of means. Statistical signicicance: NS non-significant, * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). L = linear trend of urea level, Q = quadratic trend of urea level; no significant cubic effect.

been reported also by SCHMIDT *et al.* (1978) and MOWAT *et al.* (1981). In contrast, PEPLINSKI *et al.* (1978) recorded a DM loss of 14 % during long-term storage of ammoniated high moisture corn, suggesting the bacterial activity to be the major cause for losses. SCHMIDT *et al.* (1978) observed the long-term preservative effect of urea to be better than that of ammonia when both were applied at the same level of N. In the present study, DM losses were sligthly smaller than when barley was ensiled at the moisture content of 55—60 % (HUHTANEN 1984).

The N losses slightly exceeded the DM losses, increasing with increasing moisture level ($P > 0.05$). The average loss of urea N, 27.2 % (SE 2.9 %) (Table 2), was lower than that reported by SCHMIDT *et al.* (1978). On the other hand, ØRSKOV *et al.* (1979) found only small changes in the N content of urea-preserved barley after 5 months of storage. The reason for differences in N losses of urea-treated grain might be the treatment of the sample before N analyses. In the present study, the fresh samples contained on the average 6.2 % more N than samples dried at 50 °C in vacuum. The difference in N content of fresh and dried samples was very closely related to the ammonia concentration (r 0.943). MONTGOMERY *et al.* (1980) found much higher N losses in ammonia-treated corn. Lower N losses during application and storage of urea-treated grain could explain the better long-term preservative effect of urea compared to ammonia.

A higher proportion of added urea was hydrolysed to ammonia at lower application and higher moisture levels (Table 2). Similar effects have also been found in urea-treated wheat (SCHMIDT *et al.* 1978) and straw (WILLIAMS *et al.* 1984). However, raising the levels of urea applied significantly increased the quantity of urea hydrolysed.

In the present study, the proportion of residual urea was much higher than that reported by SCHMIDT *et al.* (1978), probably because of the different ambient temperature or different urease activity of native microbiota. The proportion of ammonia N and soluble N of total N increased with increasing urea level ($P < 0.001$) and moisture content ($P < 0.001$). The urea level had no effect on the true protein concentration or solubility of barley N. SCHADEREIT *et al.* (1982) observed no differences in amino acid composition of urea-treated and dried wheat. The initial soluble N content of 3.65 g/kg DM was slightly lower than that of 4.29 g/kg DM after one year of

Table 2. Effect of urea treatment of barley on different N fractions (g/kg DM).

	Urea level				Moisture content			SEM		Statistical significance		
	1.0	1.5	2.0	2.5	22	25	32	Urea	Moisture	Urea L	Urea Q	Moisture
Total N	22.8	25.4	27.4	30.4	27.4	25.1	27.0	0.52	0.45	***	NS	*
Protein N	14.9	14.9	14.9	14.9	16.0	14.3	14.4	0.10	0.09	NS	NS	***
Residual urea N	1.14	2.30	2.93	4.55	4.52	2.43	1.24	0.86	0.75	*	NS	*
% of addition	17.8	25.3	24.4	29.2	39.8	21.5	11.3	6.3	5.5	NS	NS	*
Ammonia N	4.28	5.81	7.12	8.72	4.83	6.20	8.43	0.50	0.42	**	NS	**
% of total N	18.8	22.8	26.1	28.8	17.4	24.3	30.7	1.3	1.2	**	NS	***
% of urea N addition	54.5	50.3	45.6	43.8	40.6	47.6	57.8	0.83	0.72	***	NS	***
N loss of urea N	27.4	24.1	29.9	27.0	19.7	30.9	30.9	5.4	5.9	NS	NS	NS
Soluble N	9.6	12.0	14.7	17.4	13.4	12.2	14.9	0.22	0.22	***	NS	***
% of total N	42.2	47.2	53.5	58.3	48.3	48.0	54.6	0.63	0.55	***	NS	***
Soluble N of barley[1]	4.20	3.87	4.62	4.47	4.08	3.57	5.22	0.04	0.03	NS	NS	*

[1] Soluble N — ammonia N — urea N. For statistical significance; see Table 1.

storage. At the highest moisture level the soluble N content tended to be higher than at the lower moisture level.

The average temperatures of treated grain showed an initial rise for few days after

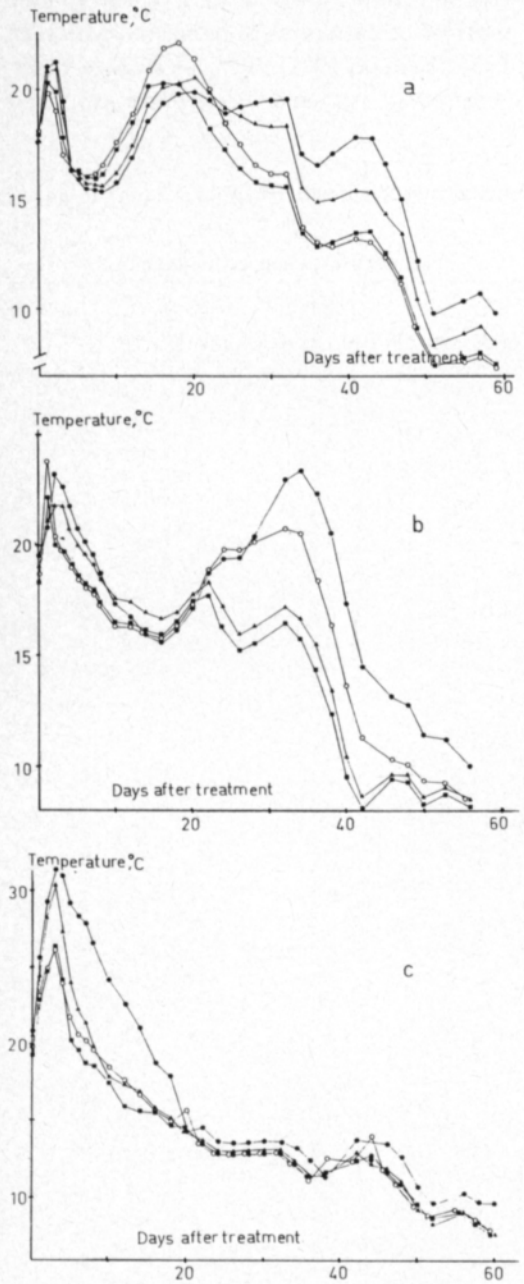

Fig. 1. Effect of urea level on temperature in barley preserved at moisture content of 22 % (a), 25 % (b) and 32 % (c). Urea level: 1.0 % ● ; 1.5 % ▲ ; 2.0 % ■ ; 2.5 % ○.

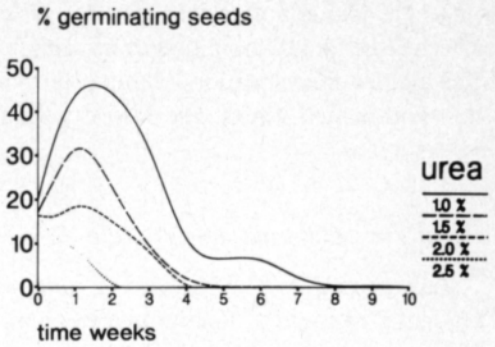

Fig. 2. Effect of four levels of urea application on the percentage of germination of barley seeds during 10 first weeks of storage.

treatment (Fig. 1). At the lowest urea level the temperature rise was slightly higher, probably indicating higher microbial activity. After 1—2 weeks of the treatment the temperature began to rise again in the silos preserved at 22 and 25 % moisture contents. The reason for the rise in temperature might be the hydrolysis of urea to ammonia and carbon dioxide, which is a heat generating reaction. Also the chemical reaction of ammonia with grain (BOTHAST *et al.* 1975) might explain the rise temperature to some extent. In silos preserved at 32 % moisture content there was no secondary rise in temperature, probably due to the higher rate of hydrolysis of urea and thus higher initial ammonia concentration. At all three moisture levels the temperature was highest at 1.0 % urea level. This urea level was probably insufficient to produce enough ammonia to eliminate microbial activity. After the initial rise the grain temperatures declined, thereafter generally reflecting ambient temperatures except for the silo treated with 1.0 % of urea at 32 % moisture level. In this silo, the temperature rose later 5—7 °C above ambient temperature, and the growth of mould could be seen.

The germination of untreated seed lots was low, about 20 %, since the examination was made soon after harvesting. Urea treatment stimulated germination for one week after treatment, but germination was inhibited rapidly after 1—4 weeks following treatment

(Fig. 2). The concentration of urea clearly affected the rate of inhibition of germination. The highest concentration inhibited germination within two weeks, the lowest within 8 weeks.

Fungi and actinomycetes on grain

Representatives of 33 genera of fungi and unidentified species of actinomycetes mainly belonging to the genus *Streptomyces* were present in the seed lots. Bacteria other than actinomycetes were not analysed. Most of the fungi were field fungi (CHRISTENSEN and KAUFMANN 1965), mainly *Acremonium, Alternaria, Bipolaris, Cladosporium, Fusarium* and *Harzia* (Table 3). These are frequent inhabitants of cereal grain (MALONE and MUSKETT, 1964, YLIMÄKI 1981). Typical storage fungi, *Aspergillus* ssp. and *Penicillium* ssp. were

Table 3. The occurrence of fungi and actinomycetes on untreated and urea-treated barley grain during 12 months of storage.

Fungus	Untreated grain	Time after urea application (months)			
		0—3	4—6	7—9	10—12
	% of seeds contaminated (+ = less than 0.1 %)				
Absidia van Tieghem	0	+	0	0	0
Acremonium Link:Fr.	39.7	2.7	+	+	0
Alternaria Nees:Fr.	83.3	2.8	0	+	0
Arthrinium Kunze:Fr.	0	+	0	0	0
Aspergillus Mich.:Fr.	0	4.6	3.2	2.6	+
Bipolaris Shoemaker	12.7	1.0	0	0	0
Botryotrichum Sacc. & March.	0	+	+	+	+
Ceratocystis Ellis & Halst.	5.2	+	0	0	0
Chaetomium Kunze:Fr.	0	+	0	+	+
Chrysosporium Corda	0	0	0	+	0
Cladosporium Link:Fr.	54.8	+	0	0	0
Doratomyces Corda	0	+	0	0	0
Drechslera Ito	+	+	0	0	0
Epicoccum Link:Schlecht.	2.2	+	0	0	0
Fusarium Link:Fr.	18.7	9.6	1.6	+	2.5
Fusidium Link:Fr.	4.7	+	0	0	0
Gliocladium Corda	+	+	+	0	0
Gonatobotrys Corda	+	0	0	0	0
Graphium Corda	0	+	0	+	0
Harzia Cost.	10.5	+	0	+	0
Humicola Traaen	0	0	0	+	0
Mortierella Coemans	0	+	0	0	0
Mucor Mich.:St.-Am.	2.2	+	+	+	+
Papulaspora Preuss	0	0	0	+	3.2
Penicillium Link:Fr.	+	1.7	+	+	+
Peziza L.:St.-Am.	0	0	0	1.3	0
Phoma Sacc.	0	+	0	0	+
Scopulariopsis Bain.	0	67.4	97.3	89.1	35.8
Stilbum Tode:Fr.	0	+	0	0	0
Trichocladium Harz	0	0	0	+	0
Trichoderma Pers.:Fr.	0	0	0	+	+
Trichothecium Link:Gray	+	0	0	+	0
Ulocladium Preuss	+	+	0	0	0
Verticillium Nees:Link	+	+	0	0	0
unidentified genera	+	+	+	+	+
Bacteria: actinomycetes	11.8	45.2	20.2	29.6	34.7

Table 4. Effect of urea concentration of the grain on the percentage of barley seeds contaminated with fungi and actinomycetes during 12 months of storage.

Fungus	Urea concentration control[1]	1.0 %	1.5 %	2.0 %	2.5 %	Counts not independent of urea (G-test, SOKAL and ROHLF 1969)	
		% of seeds contaminated (+ = less than 0.1 %)				Urea vs. control[2]	Urea concentrations[3]
Acremonium spp.	39.7	3.5	3.9	0.8	0.3	***	*
Alternaria spp.	83.3	5.9	1.4	0.1	+	***	**
Aspergillus spp.	0	11.2	1.6	1.2	0.5	***	***
Bipolaris sorokiniana	12.7	1.6	0.6	0.1	+	***	NS
Cladosporium spp.	54.8	0.2	+	+	+	***	NS
Fusarium spp.	18.7	15.5	6.3	4.2	1.3	***	***
Penicillium spp.	0.2	3.3	0.8	0.5	0.3	NS	NS
Scopulariopsis brevicaulis	0	71.3	72.2	67.9	65.3	***	NS
Scopulariopsis spp.	0	20.1	27.7	32.9	37.2	***	**
Actinomycetes	11.8	52.3	42.4	33.2	33.0	***	***
Number of seeds examined	600	10 800	10 800	10 800	10 800		

[1] Control seeds were not stored; the effect of storage and urea-treatment cannot be partitioned.
[2] Sums of all urea treatments are tested against controls.
[3] All four treatments are compared with each other. Zero counts were replaced with 10^{-50} to enable ln transformations.

present on less than 5 % of seeds. The most commonly encountered fungi after urea treatment were *Scopulariopsis* ssp. (Table 4).

The indirect method for detecting fungi on seeds merely reflects the potential ability of certain fungal spores to germinate in adequate conditions. The activities of microbes in actual storage silos compared to those on Petri dishes must be interpreted with caution. The decline of certain fungi in the blotter test indicates the death of fungal propagules in storage silos. The detected fluctuations and increasement of some fungi is probably an expression of these fungi having reproductive or metabolically active phases during storage.

The grain stored at the highest moisture level (32 %) contained more fungi and less actinomycetes than other grain lots. Especially *Fusarium* ssp. occurred frequently in moist grain. High moisture at harvesting favoured the occurrence of *Fusarium* ssp. (YLIMÄKI 1981). High counts of *Fusarium* ssp. in moist grain are probably due to the late harvesting date of the seed lot.

The number of fungal genera on grain declined after all urea treatments during storage. Only three of the initial 17 genera in untreated controls were present after 10 months of storage. The decline was much faster than that reported of untreated seeds heat-dried to a moisture content of 14 % (CHRISTENSEN and KAUFMANN 1965). However, the decline was not as rapid as reported for urea-treated hay by HLÖDVERSSON and KASPERSSON (1986) or ammonia-treated corn by BOTHAST *et al.* (1973, 1975) and MONTGOMERY *et al.* (1980). High urea concentrations (2.0 and 2.5 %) accelerated the decline of fungi.

After urea treatment there were 16 genera of fungi not detected in untreated seed lots. Most of them were found only occasionally during the first three months of storage, and their presence is apparently due to the greater number of seeds examined compared to the untreated controls. *Scopulariopsis* ssp. were the only fungi showing a rapid increase after urea treatments. In certain seed lots the total number of *Scopulariopsis* ssp. exceeded

100 %, one seed often being occupied by more than one species (Table 4). Urea and ammonia treatments have shown to increase the number of *Scopulariopsis* ssp. on different plant materials (Bothast *et al.* 1973, 1975, Montgomery *et al.* 1980, Hlödversson and Kaspersson 1986). The vigorous growth of *Scopulariopsis* ssp. on seeds may be due to underestimation of counts of certain other fungi not cabable of competing with *Scopulariopsis*. In addition, the counts of actinomycetes increased after urea treatments, especially at the lowest urea concentration. Also ammonia has been observed to increase the number of actinomycetes on corn (Bothast *et al.* 1975).

Aspergillus ssp.

Aspergillus ssp. were not found in untreated seed lots. After urea treatment they were prevalent in seed lots of the highest and lowest moisture contents. At urea concentrations exceeding 1.5 % *Aspergillus* ssp. were found only occasionally. At the lowest urea concentration (1.0 %) the percentage of contaminated seeds started to rise one week after treatment and reached the maximum of 27 % within six weeks, declining slowly towards the end of the storage period (Fig. 3).

Aspergillus ssp. are frequently reported on stored grain and they have been found to be viable still after seven years of storage. Several species are known for their ability to produce a wide variety of chemical substances including mycotoxins (Malone and Muskett 1964, Raper and Fennel 1965, Domsch *et al.* 1980). The most dangerous species, *A. flavus* Link:Gray, was not found in the present study, and sufficient urea treatment seemed to reduce contamination of *Aspergillus* ssp. efficiently.

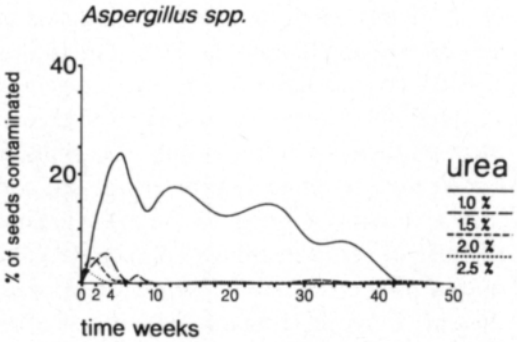

Fig. 3. Occurrence of *Aspergillus* ssp. on barley seeds during storage at four levels of urea application.

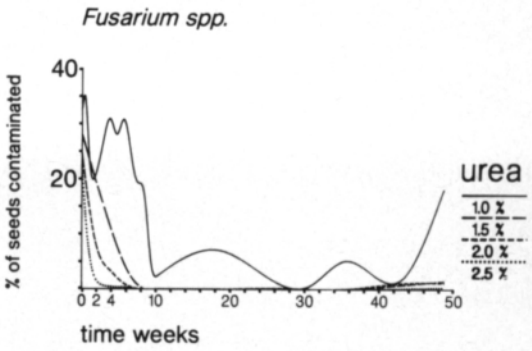

Fig. 4. Occurrence of *Fusarium* ssp. on barley seeds during storage at four levels urea application.

Fusarium ssp.

Fusarium species occurred most frequently in seed lots of the highest moisture content and lowest urea concentration. Urea concentration of 2.5 % reduced the percentage of contaminated seeds to zero within 4 weeks and 1.5—2.0 % within 7 weeks. However, there was a slight increase of contaminated seeds towards the end of storage (Fig. 4). The predominant species were *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Sm.) Sacc. and *F. poae* (Pk.) Wr.

Fusarium species are common inhabitants of Finnish grain especilly after rainy harvest season. (Uoti and Ylimäki 1974, Ylimäki 1981). They are destructive plant pathogens which can produce mycotoxins such as trichotocene (T-2 toxin, diacetoxyscirpenol), zearalenone and vomitoxin before harvesting and during storage (Joffe 1974, Neish *et al.* 1982). Estrogenically active zearalenone has been detected in undried seed samples containing *Fusarium* species, while dried samples containing *Fusarium* ssp. were free from me-

tabolites of fungi. Seed lots containing zearalenone were heavily contaminated (YLIMÄKI *et al.* 1979). In the present study, sufficient application of urea probably eliminated the risk of mycotoxin production by *Fusarium* ssp., while the percentage of contaminated seeds rapidly declined to less than 5 % (Fig. 4). On the other hand, ammonia treatment has been shown to inactivate zearalenone in grain (MÜLLER 1983).

Scopulariopsis ssp.

Scopulariopsis ssp. were the most frequent fungi in all seed lots after urea treatments. Fungi were not present on untreated grain. *S. brevicaulis* was the first invader of the seeds. During the first two weeks of storage there was a rapid increase of *S. brevicaulis* (Sacc.) Bain in all urea-treated grains and the percentage of contaminated seeds remained high for 40 weeks until a rapid decline towards the end of storage. The highest urea concentration appeared to delay the increase and fasten the decline of the fungus (Fig. 5). The percentage of *Scopulariopsis* ssp. other than *S. brevicaulis* began to increase after one week's storage, reaching a peak within 18 to 40 weeks of storage, depending on the urea concentration: the higher the concentration, the higher the incidence of *Scopulariopsis* ssp. (Fig. 6).

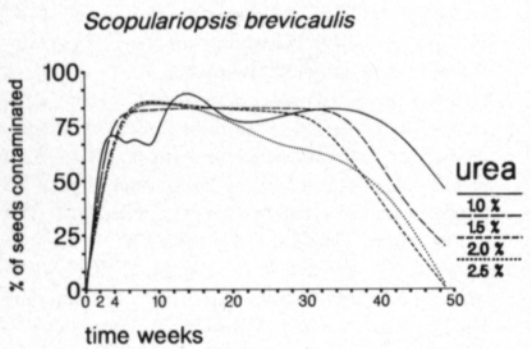

Fig. 5. Occurrence of *Scopulariopsis brevicaulis* (Sacc.) Bain on barley seeds during storage at four levels of urea application.

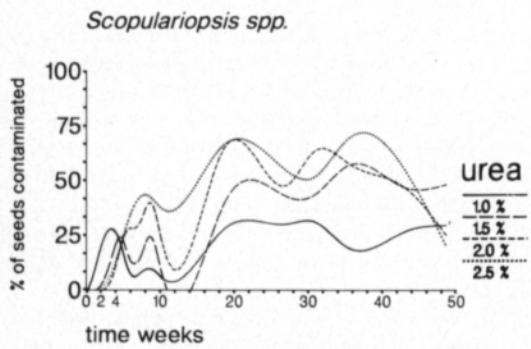

Fig. 6. Occurrence of *Scopulariopsis* ssp. other than *S. brevicaulis* on barley seeds during storage at four levels of urea application.

Scopulariopsis ssp. are distributed worldwide. They have been reported on plant debris, numerous organic materials and soils (DOMSCH *et al.* 1980). They are not typical seed contaminants (MALONE and MUSKETT 1964, YLIMÄKI 1981).

High counts of *S. brevicaulis* as well as other species have been reported on ammonia-treated corn (BOTHAST *et al.* 1973, 1975, MONTGOMERY *et al.* 1980) and urea-treated hay (HLÖDVERSSON and KASPERSSON 1986). The optimal growth is reported at pH 7—8 and above. The fungi tolerate temperatures ranging from +5 °C to 37 °C; they are relatively xerophilic and they can decompose, utilize and tolerate a wide range of different organic and inorganic compounds (DOMSCH *et al.* 1980). *S. brevicaulis* is encountered as a parasite causing onychomycosis in man, and dermatomycosis of feet and other parts of body (RAPER and THOM 1949, ONIONS 1966). Possible risks for human health caused by *S. brevicaulis* in urea-treated grain warrant further investigation.

In conclusion, the results of the present study show that urea is an effective and cheap preservative of high moisture grain. In certain feeding conditions when the supply of rumen degradable nitrogen is inadequate, the additional nitrogen may be useful for rumen microbes. Application of urea, 2.0 % or more on fresh weight basis, eliminated the growth of fungi, especially those producing mycotoxins.

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SELOSTUS

Urea rehuviljan säilöntäaineena. Säilöntäominaisuudet ja vaikutus homeiden kasvuun

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Tutkimuksessa selvitettiin urean annostelutason (1.0, 1.5, 2.0 ja 2.5 % tuorepainosta) vaikutusta eri kosteuspitoisuuksissa (22, 25 ja 32 %) puidun ohran säilönnässä. Urea lisättiin ohraan vesiliuoksena (1:1) hapotuslaitteella viljansiirtoruuvin alkupäähän ja ohra säilöttiin 400 litran lämpöeristettyihin koesiiloihin aerobisesti. Viljan kemiallinen koostumus analysoitiin ennen säilöntää ja 12 kuukauden kuluttua säilönnästä.

Ureatason ollessa 1.5 % tai korkeampi ohra säilyi laadultaan hyvänä koko varastointiajan. Ainoastaan 32 %:n kosteudessa 1.0 %:n ureatasolla säilötty ohra pilaantui 8 kuukauden varastoinnin jälkeen. Urean säilöntävaikutus perustuu ammoniakkiin, jota muodostuu urean hydrolysoituessa pääasiassa mikrobien tuottaman ureaasin vaikutuksesta. Hydrolysoituneen urean määrä lisääntyi viljan kosteuspitoisuuden ja annostelutason lisääntyessä. Toisaalta hydrolysoituneen urean osuus näytti kuitenkin laskevan annostelutason lisääntyessä. Ureakäsitellyn viljan lämpötila nousi säilönnän jälkeen 17—20 °C:sta 22—23 °C:seen. Keskimääräinen kuiva-ainetappio oli 2.9 % ja raakavalkuaistappio 6.1 %.

Ureakäsittelyn vaikutus ohran kemialliseen koostumukseen oli vähäinen. Merkittävin muutos oli raakavalkuaispitoisuuden nousu sekä ammoniakki- ja liukoisen typen osuuden lisääntyminen. Maitohappokäymistä ei todettu, mutta etikkahappoa muodostui jonkin verran (0.6—3.5 g/kg kuiva-ainetta).

Homesienten esiintyminen urealla käsitellyissä ohrarissä selvitettiin idättämällä siemennäytteet petrimaljoissa kostutetulla suodatinpaperilla. Siementen itävyys todettiin 10 vrk:n idätysajan jälkeen. Siementen pinnalla esiintyneet homeet tunnistettiin n. 3 viikon idätysajan kuluttua stereo- ja valomikroskoopin avulla. Kullakin näytteenottokerralla tutkittiin 200 siemenen näyte-erä jokaista eri kosteus- ja ureatasoa edustavasta ohrasiilosta. Yhteensä tutkimuksen kuluessa määritettiin 43800 siemenen homelajisto.

Siemeneristä tavattiin yhteensä 33 sienisuvun edustajia sekä tarkemmin määrittämättömiä sädesieniä. Useimmat sienisuvut esiintyivät näytteissä satunnaisesti ja niiden määrä väheni varastoinnin aikana. Kosteimpana (32 %) varastoidussa ohrassa esiintyi enemmän homeita ja vähemmän sädesieniä kuin muissa sienierissä. Kaikki ureakäsittelyt vähensivät homesienten määrää siemenissä.

Käsittelemättömissä siemenissä yleisimmät sienisuvut, *Acremonium, Alternaria* ja *Cladosporium,* tuhoutuivat siemenistä nopeasti ureakäsittelyn jälkeen. Punahomeita (*Fusarium*-lajeja), jotka varsinkin kosteana varastoidussa viljassa saattavat muodostaa haitallisia homemyrkkyjä, esiintyi käsittelemättömissä siemenerissä varsin runsaasti. Niiden määrä oli suurin kosteimpana puidussa ohraerässä. Alhaisimmat ureatasot eivät vähentäneet punahomeiden määrää kovin nopeasti. Ureapitoisuuden ollessa vähintään 2 % myöskin punahomeet tuhoutuivat noin 2 viikon varastoinnin aikana.

Tyypillisiä varastohomeita, *Aspergillus*-lajeja, siemenissä esiintyi vähän ja yli 1.5 %:n ureapitoisuus esti niiden lisääntymisen varastoinnin aikana. Kaikki ureakäsittelyt lisäsivät *Scopulariopsis*-sienten, joita ei esiintynyt käsittelemättömissä siemenissä, määrän hyvin suureksi. *Scopulariopsis*-sieniä esiintyi toisinaan yli 100 %:ssa siemenistä, sillä samassakin siemenessä saattoi esiintyä useita eri lajeja. *Scopulariopsis*-sienet alkoivat lisääntyä hyvin nopeasti toisen varastointiviikon aikana ja niiden määrä saavutti huippunsa noin 4 viikon varastoinnin jälkeen. Sienten määrä pysyi hyvin suurena 35—40 viikkoa, mutta väheni nopeasti viimeisen 10 viikon aikana. Korkeimmat ureapitoisuudet näyttivät nopeuttavan *Scopulariopsis*-sienten vähenemistä varastoinnin lopussa. Yleisin laji oli *S. brevicaulis,* jonka tiedetään aiheuttavan ihmisille ihotauteja. Myöskin sädesienten määrä lisääntyi ureakäsit-

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telyn seurauksena kaikissa siemenerissä. Niillä tiedetään olevan osuutta homepölykeuhkon synnyssä.

Tämän tutkimuksen perusteella urea soveltuu hyvin kostean viljan säilöntään ja on lisäksi kustannuksiltaan edullinen. Käytettäessä heinää tai olkea karkearehuna urealla säilötyllä viljalla voi lisäksi olla merkitystä pötsimikrobiston typen lähteenä. Ureatason ylittäessä 2 % useimpien homesienten, erityisesti homemyrkkyjä muodostavien, kasvu estyi lähes kokonaan.