Sensitivity of Fungi to Urea, Ammonium Nitrate and their Equimolar Solution UAN

Karel VEVERKA, Jindra ŠTOLCOVÁ and Pavel RŮŽEK

Department of Mycology, Division of Plant Medicine, Crop Research Institute, Prague-Ruzyně, Czech Republic

Abstract

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The sensitivity of oomycota, saprophytic and pathogenic fungi to urea, ammonium nitrate and UAN (urea plus ammonium nitrate in equimolar solution) was studied in laboratory trials. The compounds were applied in agar in concentrations of 0.06, 0.19 and 0.6M. The most toxic was urea. Ammonium nitrate inhibited the growth of fungi only in higher concentrations. In contrast, the growth of *Gaeumannomyces graminis* was stimulated by even the highest concentration of 0.6M ammonium nitrate. The fungi most sensitive to urea and UAN were *Alternaria tenuissima*, *Botrytis cinerea*, *Cladosporium cladosporioides* and *Pseudocercosporella herpotrichoides*. No synergistic effect between the two compounds in UAN was found. Urea was toxic also to *Colletotrichum acutatum* which does not produce urease. Likewise, the urease inhibitor NBPT did not decrease the toxicity of urea to fungi; the urea degradation product ammonia should, therefore, not be assumed to be the only toxic agent. Application of urea in agricultural practice can decrease the population of a pathogen not only by the stimulation of antagonists, but also by the direct toxic effect. The tested concentrations of 0.06–0.6M correspond to 0.36–3.6% (w/w) solution of urea and to 0.64–6.4% UAN used in agricultural practice as a 75% water solution. If the dilution and metabolisation under natural conditions is taken into account, the concentration of urea 0.06M (0.36%) was too low to have an effect of practical importance on fungi. While after application of urea on plants or on plant debris its concentration is increasing due to water evaporation, the concentration of the extremely hygroscopic UAN is decreasing. Therefore, the control effect will depend more on the applied rate than on the concentration.

Keywords: urea; ammonium nitrate; UAN; fungi; urease inhibitor NBPT

The nutrients in both inorganic and organic fertilisers are able to influence the incidence and severity of biotic plant diseases, pests and weeds populations and their impact on the crop. Most of the information on this aspect deals with the effect of nutrients via plant. Individual elements have different roles; in general it can be said that they change the losses caused by pests by influencing plant resistance, alter plant growth and in this way the microclimate in the stand. Increased nutrition used to be prescribed as the first measure to control plant diseases. The most important aspect of this is an increase in the ability of the crop to compensate the losses. The effect of indi-

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vidual nutrients is very complex – the severity of one plant disease can be decreased, that of others increased (Huber 1980). It is generally accepted that calcium increases plant resistance, whereas high rates of nitrogen increase the populations of aphids, acari and the incidence of many diseases (Wermelinger *et al*. 1985).

A completely different question is the direct effect of the fertilisers on plant pathogens and pests. Information on this is very scarce, with the exception of calcium cyanamide. High rates of urea control *Synchytrium endobioticum* (Schilb.) Percival in the soil (Tarasova & Beskorovajnyj 1973). This method is not ecologically acceptable nowadays, because of the extremely high rates of urea applied. Urea is effective in the control of *Phellinus noxius* (Corn.) G. H. Cunn. which causes brown root disease that is responsible for damage to numerous orchard and forest tree species in the tropics (Chang & Chang 1999). Several fungicides were tested, but did not decrease the survival of this fungus. On the other hand, 3000 ppm of urea or 400 ppm of NH_3 completely killed the fungus. Ammonia is supposed to be the active agent released from urea. It is toxic to the fungus, increases the pH, and most probably increases the microbial antagonism that reduces survival of the pathogen (Setua & Samaddar 1980).

An intermediate compound of the hydrolysis of urea in the soil is ammonium carbonate. Homma *et al.* (1981a, b) successfully used sodium bicarbonate in the control of citrus storage diseases and cucumber powdery mildew. Volatile NH_3 was also lethal to other root rotting fungi (*Ganoderma australe* (Fr.) Pat. 1890, *G. lucidum* (Curtis) P. Karst. 1881, *G. tropicum* (Jungh.) Bres. 1910, *Rigidoporus vinctus* (Berk.) Ryv., *Heterobasidion annosum* (Fr.) Bref. 1888 and *Rosellinia necatrix* (Hart.) Berk.) (Chang & Chang 1999; Johanson *et al.* 1998).

Urea is able to reduce populations of certain soilborne fungi through NH_3 release upon hydrolysis, e.g. *Phytophthora* sp., *Pythium ultimum* Trow 1901, *Thielaviopsis basicola* (Berk. et Br.) Ferraris 1912 and *Macrophomina phaseolina* (Tassi) Goid. 1947 (Tsao & Oster 1981; Chun & Lockwood 1985).

Another question is the effects of liquid fertilisers, which may directly hit pests and pathogens at spraying. Raja & Kurucheve (2000) studied the farmers' experience that sheep excrements control sheath blight of rice. Sheep urine in a 10% concentration prevented the growth of *Rhizoctonia solani*, production and germination of sclerotia *in vitro*. Seed treatment with sheep urine enhanced seed germination and vigour of paddy seedlings.

Ammonium sulphate and UAN (urea plus ammonium nitrate in equimolar solution) are often used as spray additives which increase the activity of some pesticides and allow to decrease the hectare rates, e.g. of growth herbicides or glyphosate. UAN alone was toxic not only for the model object *Tribolium confusum* Jacq. du Duval but also for *Meligethes aeneus* (Fabr.), *Leptinotarsa decemlineata* Say, *Tetranychus urticae* Koch and for the predator *Coccinella septempunctata* L. None of the other tested liquid fertilisers showed insecticidal activity. Most important from the practical point of view is the effect of UAN on *M. aeneus* because it is applied in winter rape at the time of invasion by the pest. UAN in mixtures with insecticides has a synergistic effect against *M. aeneus* (Oliberius & Veverka 1985; Veverka & Oliberius 1985). Solutions of only urea or ammonium nitrate had no insectidal activity. It means that the insecticidal activity of their equimolar solution is a synergistic effect of both compounds. The mode of action is unknown. We suppose that a decisive factor is the extremely high hygroscopicity of UAN which prevents the spray droplets to dry and in this way enables increased transcuticular penetration by the compounds. UAN also had a synergistic effect in comparison with urea or ammonium nitrate activity alone against *Agrobacterium tumefaciens* (Smith and Townsend 1907) Conn 1942, *Xanthomonas campestris* pv. *vesicatoria* (Dodge 1920) Dye 1978 and *Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900. No synergistic effect was detected against *Erwinia chrysanthemi* pv. *chrysanthemi* Burkholder, McFaden and Dimock 1953 and *Corynebacterium michiganense* pv. *insidiosum* (McCulloch 1925) Dye and Kemp 1977, which were also highly sensitive to the single compounds (Veverka *et al*. 1988).

The aim of our work was to explore the sensitivity of a range of fungi to urea and ammonium nitrate and to find out if there is any synergistic effect of urea and ammonium nitrate (UAN) against fungi.

MATERIALS AND METHODS

The tested fungi are listed in Table 1. They were grown on malt agar at pH 6.8, and cultures incubated at room temperature 21–24°C. Urea,

Table 1. Colony diameter of tested fungi after 4 days – control variants

	Colony diameter (mm)
Chromista – oomycota	
Pythium ultimum Trow 1901	87
Pythium debaryanum Hesse 1874	83
Fungi – anamorphic fungi	
Alternaria tenuissima (Kunze ex Pers.)	35
Aspergillus niger v. Tiegh. 1867	22
Botrytis cinerea Pers.: Fr.	45
Cercospora beticola Sacc. 1876	17
Cladosporium cladosporioides (Fres.) de Vries	15
Colletotrichum acutatum Simmonds ex Simmonds 1968	28
Gaeumannomyces graminis (Sacc.) v. Arx et Olivier 1952	25
Helminthosporium sp.	26
Mucor globosus Fischer 1892	71
Penicillium albidum Sopp emend. Fassatiová	14
Phoma betae Frank 1892	33
Phoma exigua var. *foveata* (Foister) Boerema 1967	40
Pseudocercosporella herpotrichoides (Fron) Deighton 1973	19
Trichoderma viride Pers.: Fr. 1794	90
Fusarium oxysporum Schlecht.: Fr. 1824 (1)	47
Fusarium oxysporum Schlecht.: Fr. 1824 (2)	46
Fusarium oxysporum Schlecht.: Fr. 1824 (3)	46
Fusarium oxysporum Schlecht.: Fr. 1824 (4)	30
Fusarium solani (Mart.) App. et Wr. (1)	27
Fusarium solani (Mart.) App. et Wr. (2)	30
Fusarium avenaceum (Fr.: Fr.) Sacc. 1886	36
Septoria nodorum (Berk.) Berkeley 1850	41
Stemphylium sp.	26
Basidiomycota – anamorphic fungi	
Rhizoctonia solani Kühn 1858	43

ammonium nitrate or urea + ammonium nitrate (UAN) in equimolar ratio were added to the medium after sterilisation in rates of 0.06, 0.19 and 0.6M (gmol/l, dilution coefficient √10). It means that the concentration of these compounds in UAN was respectively 0.03, 0.095 and 0.3M. The media were poured into Petri dishes of 10 cm diameter. Pieces of fungal mycelia (with or without spores) were transferred to the center of each dish. The growth of fungi was evaluated by the colony diameter on the fourth day after inoculation and expressed as the percentage of that in the control

free of tested substances. The trial was performed in six replications.

Another test was performed to determine whether NH_3 is in fact the active substance released from urea by the urease produced by the fungi themselves. Much like the trials described above the concentrations 0.06 and 0.6M of urea and urea with the urease inhibitor NBPT were prepared. The concentration of NBPT was in the same ratio to urea as is used in the field application, i.e. 0.006 ml NBPT per 1 g urea. Apart from representatives of fungi tested in the first trials, isolate No. 261 of *Colletotrichum acutatum* from the culture collection of the Crop Research Institute which does not produce urease (Krátká – personal communication) was included in the trial. No one of the isolates available produced urease.

Since the sensitivity of isolates of each fungal species is variable, it is not possible to precisely compare the sensitivity between individual species. Rather, our goal was to monitor general data on the sensitivity of fungi to urea. For that no statistical analyses were calculated.

RESULTS

Table 1 presents the scientific names of the tested oomycota and fungi and their growth in the control (without tested nutrients) after 4 days expressed as the colony diameter. Figures 1–3 show the relative growth, expressed in percentage, of the fungi in comparison with the control as presented in Table 1; in this manner, growth inhibition or stimulation are shown more transparently than by absolute numbers.

At the rate of 0.06M, urea inhibited the growth of almost all of the fungi more effectively than ammonium nitrate. *Botrytis cinerea* was most sensitive to urea (Figure 1). Growth inhibited at less than 50% was recorded in *A. tenuissima*, *C. cladosporioides* and *Rh. solani*. The growth of other fungi was inhibited only slightly, or not at all. In contrast, ammonium nitrate at 0.06M stimulated *A. niger* and especially *G. graminis*. No synergistic effect of the two compounds in UAN was evident. The results could be regarded as the additive effect of both compounds at half rates and in equimolar solution. Growth of none of the fungi was inhibited more by UAN than by urea alone. Growth stimulation of *A. niger* and *G. graminis* by UAN was weaker than by ammonium nitrate. *Phoma exigua*, one of the strains of *F. oxysporum* and *Stemphylium* sp. were slightly stimulated by UAN, but not by ammonium nitrate. Most sensitive to all the compounds at this rate were *T. viride* and *Rh. solani*.

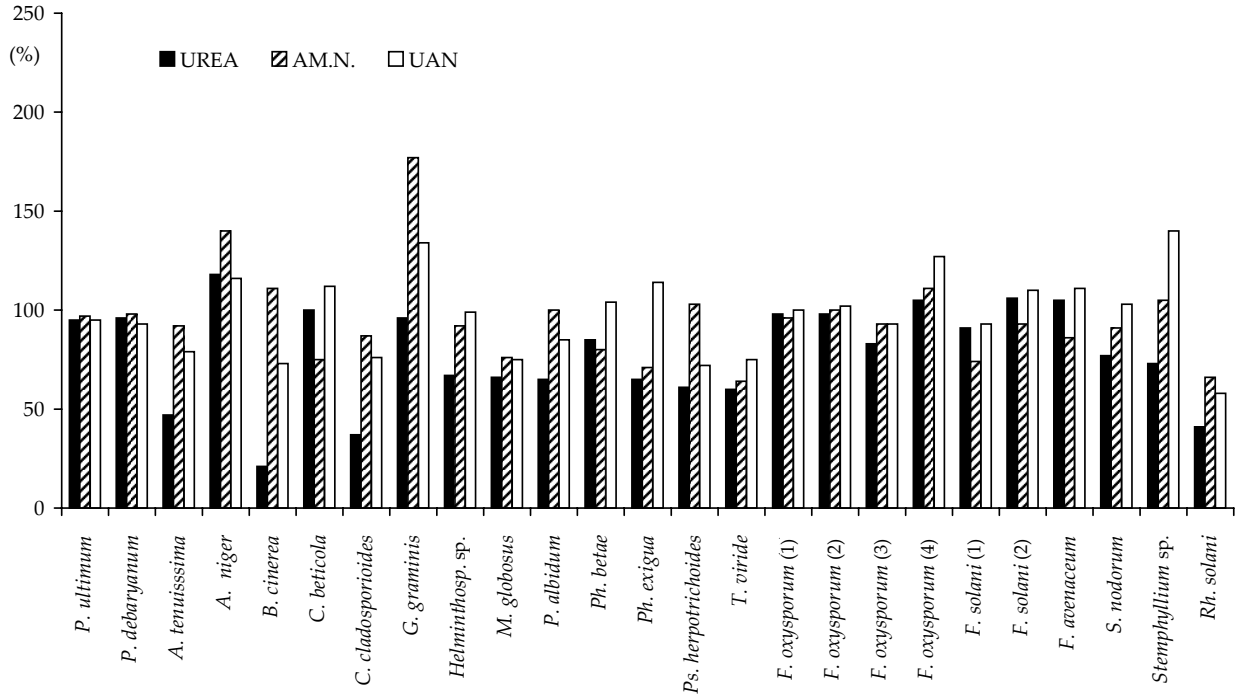

Figure 1. Growth of fungi on 0.06M urea, ammonium nitrate and UAN presented in percentage of control

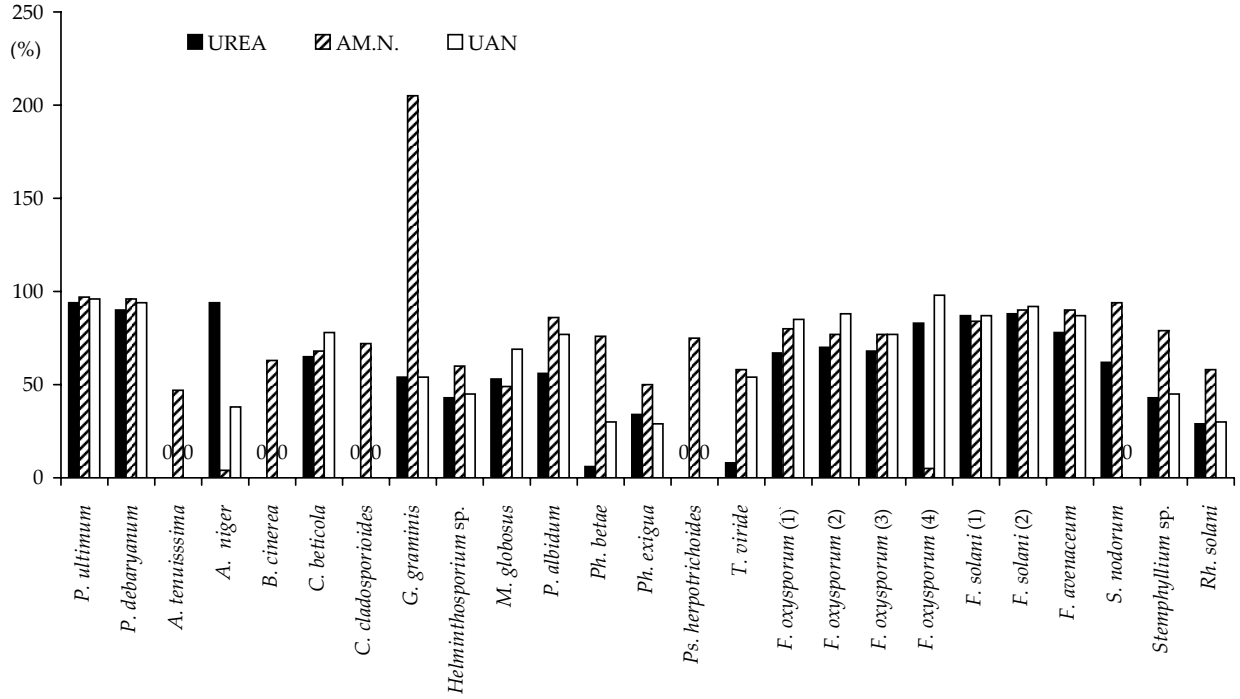

Figure 2. Growth of fungi on 0.19M urea, ammonium nitrate and UAN presented in percentage of control

The next higher rate, i.e. 0.19M of urea or ammonium nitrate, completely prevented the growth of *A. tenuissima*, *B. cinerea*, *C. cladosporioides*, *Ps. herpotrichoides* and strongly inhibited the growth of *Ph. betae*, *Ph. exigua*, *T. viride*, *Stemphylium* sp., and *Rh. solani* (Figure 2). The growth of other fungi was also inhibited more than by the rate of 0.06M. Surprising was the high stimulation of *G. graminis* by ammonium nitrate (215%) which was even higher than at the rate 0.06M (177%). Inhibition of most of other fungi was minimal.

The concentration of 0.6M of urea completely inhibited the growth of all the fungi, with exception of *F. solani*, *S. nodorum* and two of the four strains of *F. oxysporum* (Figure 3). Ammonium nitrate had the lowest effect on the growth of fungi. It stimulated the growth of *G. graminis*, but less than at its lower rates, and it had no effect on *A. niger* and *Stemphylium* sp.

There was no synergistic effect of urea and ammonium nitrate at any concentration. The fungi were not inhibited more by the UAN variant than by urea alone.

The urease inhibitor NBPT did not decrease the toxicity of urea to fungi (Table 2). *Colletotrichum acutatum* Simmonds ex Simmonds 1968, which does not produce urease, was as sensitive to urea as other fungi. Nor did the urease inhibitor NBPT decrease the toxicity of urea to *C. acutatum.*

Urea and urea + urease inhibitor were equally toxic also to *Macrophomina phaseolina* Tassi (Goid.), *P. ultimum* and *T. viride.*

Table 2. Effect of urease inhibitor NBPT on the toxicity of urea to fungi (data represents colony diameter in mm after 4 days)

Urea concentration	*C. acutatum*	*M. phaseolina*	*P. ultimum*	*T. viride*
0.6M	0	0	0	10
0.6M + NBPT	0	0	0	10
0.06M	30	25	20	100
0.06M + NBPT	31	30	23	100
Control	28	70	100	100

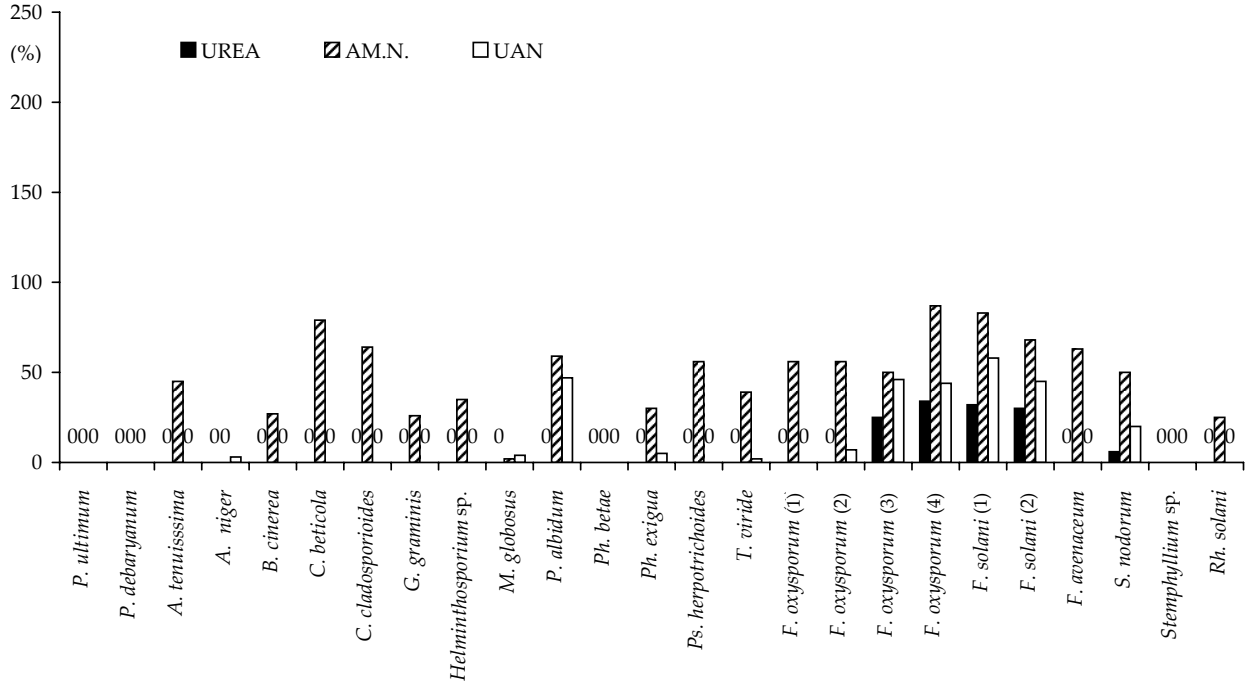

Figure 3. Growth of fungi on 0.6M urea, ammonium nitrate and UAN presented in percentage of control

DISCUSSION

Since the sensitivity of fungi to the tested substances can substantially vary among strains, the obtained data are valid for the tested strain and very approximately represent the sensitivity of each species – e.g. see the four *F. oxysporum* strains tested (Figures 1–3). The role of the form of nitrogen on disease incidence and severity under field conditions was very intensively studied in the 1960's and 1970's (Huber & Watson 1974). Bednářová (1978) discovered that growth of some strains of *G. graminis* was better on the medium with NH_4^+, while others grew better on NO_3^- as the only nitrogen source. This revealed that the situation in the field is more complex; the effect of ammonium or nitrate fertilisers on the severity of take-all disease, and maybe also on other diseases, may depend on the proportion of the strains preferring NH_4^+ or NO_3^-. Strains of *G. graminis* vary in many other traits, e.g. in the ability to use sources of carbon, vitamins etc.

The positive effect of urea on the control of fungal diseases is complex, expressing itself differently in acidic or alkaline soils (Chang & Chang 1999). Urease occurs in many bacteria, several species of yeast and a number of higher plants (Varner 1960). The production of urease in fungi is very variable (Krátká – personal communication). If we accept the assumption that the toxic agent is NH_3, the level of urea toxicity to fungi in pure cultures should depend on their own urease production. This was not confirmed in our trial (Table 2). *C. acutatum,* which does not produce urease, was inhibited by urea. Further, the urease inhibitor NBPT did not diminish the growth inhibition of fungi by urea. The mode of the toxic action by urea may be more complex.

The addition of urea to acidic soil increases the concentration of NH_3 in that soil, which may enhance the activity of soil microorganisms antagonistic to a pathogen. Volatile ammonia can reach the fungitoxic level only in alkaline soils (Chang & Chang 1999).

The high sensitivity of *Ps. herpotrichoides* to urea and UAN may be one of the reasons of the increased activity of fungicides containing benomyl if applied in mixtures with urea or UAN in early spring against eyespot disease in agricultural practice (Benada 1980). Urea sprayed in the pre-leaf fall period increases the activity of saprophytic organisms and in this way it decreases both the survival of *Venturia inaequalis* (Cooke) Wint. 1897 and the primary infections of apple trees in the following spring (Schwabe 1979). It is not known if there is also a direct toxic effect of urea on the pathogen.

Based on the presented data we reached the following conclusions:

– urea is, or produces, a toxic agent;
– ammonium nitrate is a source of nitrogen in ammonium and nitrate form;
– UAN – no synergistic effect of both its compounds to fungi was detected. Its effects combine the toxicity of urea and those of ammonium nitrate;
– application of urea in practice can decrease the populations of a pathogen not only by the stimulation of antagonists, but also by direct toxic effect. The tested concentrations 0.06–0.6M correspond to 0.36–3.6% (w/w) solution of urea and 0.64–6.4% UAN used as 75% equimolar water solution (w/w) of urea with ammonium nitrate used under trade name DAM 390. If the dilution and metabolisation under natural conditions is taken in account, the concentration 0.06M was too low to have a direct toxic effect on fungi. Higher rates should be preferred.

After application on plants or on plant debris the concentration of urea is increasing due to water evaporation, while the concentration of extremely hygroscopic UAN is decreasing. Yet a solution that does not dry up is better able to penetrate into tissues. The control effect depends more on the applied rate than on the concentration.

Liquid fertilisers never reach the same control level as modern pesticides. In agricultural practice their positive effect is restricted to special cases as e.g. mixtures with pesticides, combination of plant nutrition and pest control, or in ecological farming where urea may be better accepted for being more natural than synthetic fungicides like e.g. cuprous fungicides are. To use the positive direct toxic effect of fertilisers in practice, UAN has to be used against beetles (Veverka & Oliberius 1985) and urea against fungi.

References

Bednářová M. (1978): Příspěvek k fyziologii houby *Gaeumannomyces graminis* (Sacc.) Arx et Olivier. In: Souhrn referátů Symposia fyziologie a ekologie fytopatogenních mikroorganismů. Praha: 4–6.

Benada J. (1980): Míchání kapalných hnojiv s pesticidy. In: Kapalná hnojiva v zemědělské velkovýrobě. Sborník referátů. Kroměříž: 60–62.

Chang T.T., Chang R.J. (1999): Generation of volatile ammonia from urea fungicidal to *Phellinus noxius* in infested wood in soil under controlled conditions. Plant Pathology, **48**: 337–344.

Chun D., Lockwood J.L. (1985): Reduction of *Pythium ultimum, Thielaviopsis basicola* and *Macrophomina phaseolina* in soil associated with ammonia generated from urea. Plant Disease, **69**: 154–158.

Homma Y., Arimoto Y., Misato T. (1981a): Effect of sodium bicarbonate on each growth stage of cucumber powdery mildew fungus (*Sphaerotheca fuliginea*) in its life cycle. Journal of Pesticide Science, **6**: 201–209.

Homma Y., Arimoto Y., Misato T. (1981b): The control of citrus storage diseases by sodium bicarbonate formulation. In: Proceedings International Society of Citriculture, **2**: 823–825.

Huber D.M. (1980): The role of mineral nutrition in defense. In: Horsfall J.G., Cowling E.B.: Plant Disease – an Advanced Treatise. Vol. 5. How Plants Defend Themselves. Academic Press, New York: 381–406.

Huber D.M., Watson R.D. (1974): Nitrogen form and plant disease. Annual Review of Phytopathology, **12**: 139–165.

Johanson M., Asiegbu F., Pratt J.E. (1998): Stump treatment against *Heterobasidion annosum* with urea, possible modes of action. In: Deleatour C., Guillaumin J.J., Lung-Escarment B., Marcais B. (eds): Root and Butt Rot of Forest Trees. INRA, Paris: 40–42.

Oliberius J., Veverka K. (1985): Insekticidní toxicita dusíkatých hnojiv aplikovaných v kapalné formě. Ochrana rostlin, **21**: 301–307.

Raja J., Kurucheve V. (2000): Sensitivity of *Rhizoctonia solani* towards sheep urine. In: Total Abstract of ISR 2000. Available at http://www.nchu.edu.tw/~isr2000/total%20abstract.htm

Setua G.C., Samaddar K.R. (1980): Evaluation of role of volatile ammonia in fungistasis of soils. Phytopathologische Zeitschrift, **98**: 310–319.

Schwabe W.F. (1979): The effectiveness of benomyl and thiophanate methyl as post-harvest fungicides against apple scab fungus (*Venturia inaequalis*) in cases of benzimidazole resistance. Deciduous Fruit Grower, **29**: 474–477.

Tarasova V.P., Beskorovajnyj V.K. (1973): Komplexnyj metod borby protiv raka kartofelja. Zaščita Rastenij, **11**: 45–48.

Tsao P.T., Oster J.J. (1981): Relation of ammonia and nitrous acid to suppression of *Phytophthora* in soils amended with nitrogenous organic substrates. Phytopathology, **71**: 53–59.

Varner J. (1960): Urease. In: Boyer P., Lardy H., Myrback K. (eds): The Enzymes. Academic Press, New York: 247.

Veverka K., Kůdela V., Oliberius J. (1988): Side effects of some liquid fertilizers on phytopathogenic bacteria. Zentralblatt für Mikrobiology, **143**: 293–298.

Veverka K., Oliberius J. (1985): Synergistic insecticidal activity of urea and ammonium nitrate. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, **92**: 258–262.

Wermelinger B., Oertli J.J., Delucchi V. (1985): Effect of host plant nitrogen on the biology of two-spotted mite, *Tetranychus urticae*. Entomologia Experimentalis et Applicata, **38**: 23–28.

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Corresponding author:

Prof. Ing. Karel Veverka, DrSc., Výzkumný ústav rostlinné výroby, v.v.i., Odbor rostlinolékařství, Oddělení mykologie, 161 06 Praha-Ruzyně, Česká republika
tel.: + 420 360 851 285, e-mail: veverka@vurv.cz