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Cyanamide in plant metabolism

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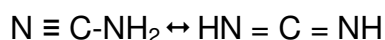E-mail: schroedl@weihenstephan.de

The calcium (Ca)-cyanamide is a nitrogen fertilizer with herbicide and fungicide side effects. Hydrogen cyanamide is absorbed as a molecule by roots and shoots of plants. It will be decomposed rapidly by the enzyme cyanamide hydratase to urea followed by urease to ammonium. From cyanamide decomposition mainly glutamate and alanine are built and the basic amino acids such as arginine, lysine and histidine are increased. The most dramatic effect of cyanamide is the strong inhibition of catalase and cytochromoxidase and the blocked thiols of redox systems. Cyanamide applied to dormant buds of fruit trees inhibits catalase activity and increases H_2O_2-content. Prior to bud break, arginine content decreases and associated with increase of polyamines such as putrescine, spermine, spermidine, histamine and cadaverine. These sharply decrease again at fully blooming stage.

Key words: Cyanamide, enzymatic break down, reaction with plant constituents and enzymes, amino acid metabolism, polyamines, dormancy break.

INTRODUCTION

The nitrogen fertilizer "Kalkstickstoff" was the first synthetic fertilizer. Through binding, the gaseous air nitrogen on calcium carbide resulted calcium (Ca)-cyanamide, which is the main nitrogen compound with about 20% N and about 50% basically reacting compounds. Calcium cyanamide is easily hydrolyzed to calcium hydroxide and hydrogen cyanamide that is present in the tautomeric forms both as amide of cyanate and nitrile of carboamide.

$$N \equiv C\text{-}NH_2 \leftrightarrow HN = C = NH$$

Cyanamide is a very reactive substance. It belongs to the classic nitriles, which are common constituents of many agricultural crops. Nitrile hydratases metabolize a rather broad spectrum of nitriles to the corresponding amides; they are frequently found in bacteria and plants (Castric et al., 1972). Lamaire and Brunel (1951) reported that the fungus *Sterigmatocystis nigra* is able to hydrolize cyanamide to urea with a "cyanamidase". Anderson (1980) and Tausig (1965) discovered a high specific "cyanase" in *Escherichia coli* bacteria.

In abiotic medium (quartz sand and water), cyanamide is constant for long time. However, after addition of small amounts of heavy metal oxides, a rapid conversion to urea takes place (Vilsmeier, 1976).

In the soil, cyanamide is decomposed both biologically and through surface catalysis on heavy metal oxides to urea and later on with urease to ammonium (Rathsack, 1955; Vilsmeier and Amberger, 1978). As a multifunctional agrochemical, it is mainly a nitrogen fertilizer however with herbicidal, fungicidal and bactericidal side effects.

MATERIALS AND METHODS

Determination of cyanamide in plants with:

1) Radioautograply ($H_2^{14}CN_2$) (Hofmann and Latzko 1954; Amberger and Latzko 1954)
2) Emissions spectroscopy ($H_2C^{15}N_2$) (Vilsmeier and Medina, 1984)
3) Photometrically with nitroprusside reagent (Hofmann and Wünsch, 1958)
4) Colorometrically with dinatrium aminopentacyanoferrate (Steller et al., 1965)
5) Isolation of the enzyme cyanamide hydratase (Stransky and Amberger, 1973)

6) Determination of amino acids with aminoanalyser (Biotronik) (Schaller and Wünsch, 1973)
7) Arginine and Arginase activity (Wünsch and Amberger, 1973)
8) Enzyme crude preparate (Amberger and Wünsch, 1963)
9) Catalase activity according to Feinstein (1949)
10) Peroxidase activity according to Purr (1950)
11) Cytochromoxidase photometrically at 550 nm extinction
12) Respiration: O_2 uptake in Warburg test apparatus

The dormancy breaking experiments were carried out by Seif El-Yazal (1990) at Konsoshem El Fayoum Research State Farm, spraying the apple trees on 17 December and pear trees on 1 January with 3% cyanamide solution ("Dormex"), each tree received 3 l. The number of floral buds from 4 tagged branches of each tree with triplicates in randomized design was counted and 2 g of each sampling were dried at 70 °C.

For analysis of polyamines, between 100 and 1000 mg of dried samples were dispersed in 10 ml of 0.1 mol/l hydrochloric acid, followed by centrifugation at 3500 g for 30 min. The supernatant was deproteinized by mixing with trichloroacetic acid to a final concentration of 20% and centrifugation at 5000 g for 15 min. The supernatant was used for chromatographic analysis. Polyamines were analyzed as corresponding dabsy derivates after pre-column derivatization using high pressure liquid chromatography (HPLC) described by Krause et al. (1995) and Henle et al. (1991).

As bud material from fruit trees was not always available, additional experiments were also carried out with turions, the rest buds of Elodea densa water lens.
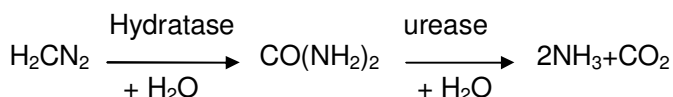
RESULTS AND DISCUSSION

Cyanamide uptake and decomposition in plants

Cyanamide is absorbed as a molecule by roots and shoots of plants. With the transpiration stream it is transported in the xylem and distributed in the whole plant system through the symplast. When applied in higher concentrations, it accumulates mainly in the cell vacuoles of leaves and can cause necrotic damages and plant death. The herbicidal effect is closely restricted to cyanamide excluding decomposition products. When applied in sublethal doses, after a few hours it can be identified in all parts of the plants with radioautography ($H_2{}^{14}CN_2$), emission spectroscopy ($H_2C^{15}N_2$) and photometrically.

A few hours after stopping the cyanamide supply, it could not be detected anymore because it is rapidly metabolized. Sometimes, noticed traces of dicyandiamide are mainly a matter of impurity of the cyanamide sample; dimerization can happen only under alkaline conditions (Rathsack, 1955).

In the early experiments of Hofmann et al. (1954a, b), the breakdown of cyanamide in plants was studied with a crude enzyme preparate (Amberger and Wünsch, 1963) from potato or alfalfa leaves and soybean determining the end-products NH_3 and CO_2. Urea could be identified only in traces. Neither the addition of crystallized urease nor urease inhibition with hydrochinon did diminish cyanamide content, speculating the idea, that two enzymes could be involved in cyanamide decomposition. They argued that most crop plants are able to decompose cyanamide enzymatically. The separation of these combined enzymes succeeded that of Stransky and Amberger (1973) with the soil fungus *Myrothecium verrucaria* grown on cyanamide as the only nitrogen source. Through continual fractionation of the crude enzyme extract with increasing concentration of ammonium sulphate, we could isolate and identify the very active and strong specific enzyme cyanamide hydratase which converts cyanamide rapidly and quantitatively to urea and subsequently with urease to ammonia and CO_2. The enzyme is specifically induced by cyanamide and reaches full activity after 48 h.

$$H_2CN_2 \xrightarrow[+\ H_2O]{\text{Hydratase}} CO(NH_2)_2 \xrightarrow[+\ H_2O]{\text{urease}} 2NH_3 + CO_2$$

In Vitis cuttings fed with $H_2{}^{14}CN_2$, one third of the total ^{14}C activity was released as CO_2 after 20 h and 50% after 72 h chase (Goldbach et al., 1988). Even if the enzyme cyanamide hydratase has not yet been isolated and purified from plants (!) up to now, its activity induced by the substrate cyanamide could be determined in many experiments (not noticed here) with crude enzyme preparates. Urea as an intermedial product could only be noticed in traces due to a high urease activity. Hartmann et al. (1991, 1992) have purified the cyanamide hydratase from the verrucaria fungus to homogeneity, characterized the amino acid sequence and elucidated its gene structure. Maier-Greiner et al. (1991a, b) succeeded to transfer this gene into plants. Thus, cyanamide as a selective agent and cyanamide hydratase from *M. verrucaria* as a marker gene are used for transformation of wheat (Wecks et al., 2000), arabidopsis, potato, rice and tomato (Damm, 1998; Zhang et al., 2005) and soybean (Ulanow and Widholm, 2007). The transformed cyanamide hydratase expressing plants are able to convert cyanamide intensively to urea. No abnormalities in metabolism and no visible damages could be noticed, however, the free amino acids accumulated more than 4 fold compared with untransformed plants indicating a rapid synthesis. No doubt, the implantation of the cyanamide hydratase gene, isolated from *M. verrucaria* into crop plants making them cyanamide tolerant is a great scientific success. However, the very increased enzymatic breakdown of cyanamide can exceed the NH_3 detoxification capacity when high amounts of the Ca-cyanamide fertilizer are applied especially to germinating and young growing seedlings that are poor on carbohydrate skeletons for amino acids synthesis. Cyanamide is toxic to uninduced *Myrothecium* fungus as well as to plants.

The chemically very reactive cyanamide has long been considered as an industrial product not occurring in the nature. Japanish researers, Kamo et al. (2003, 2006a, b) succeeded to verify the biosynthesis of cyanamide *de novo* in two *Vicia* species (villosa and cracca) acting as

Table 1. Amino acid content of barley seedlings fed with cyanamide (50 mg N/l nutr. solution).

Amino acids	Exposure time (h)							
	6		**12**		**24**		**30**	
	Cy	Control	Cy	Control	Cy	Control	Cy	Control
Alanine	++	+	++	+	+++	+	++	+
Leucine	++	+	++	+	++	+	++	+
Valine	++	+	++	+	++	+	++	+
Glutamate	++	+	++	+	++	+	++	+
γ-aminobutyrate	++	+	++	+	++	+	++	+

+, Low; ++, higher; Control, $Ca(NO_3)_2$; Cy, cyanamide.

an allelochemical while more than one hundred other plant species tested contained no detectable cyanamide. The legume *Vicia villosa* is used in USA as a winter crop in nontillage farming system for its weed suppressing peculiarity and in Japan for "soil cleaning" of abandoned rice fields.

Cyanamide metabolism

Apart from the enzymatic breakdown of cyanamide, there are some direct effects of this highly reactive substance with thiol-, amino-, hydroxyl- and carboxyl groups, well documented by Michaud (1979).

The water thyme, Elodea densa, exhibits the light dependant peculiarity to hyperpolarize the electrochemical potential difference of membranes. This reaction is stopped already a few hours after 5 mM cyanamide exposure (Blaser, unpublished) and follows with a high significant efflux of K^+ increasing from 25 to 100 mM cyanamide concentration (Burger, 1989). This loss of hyperpolarization and the enhanced permeability of membranes can be explained with the oxidation of membrane lipids with high H_2O_2-concentration caused by the catalase inhibition of cyanamide (Elstner, 1990).

In all our experiments, we could never discover the amino acid cysteine in cyanamide fed plants, because cyanamide already in low concentration reacts rapidly and nonenzymatically with the thiol group of cysteine forming guanidine and isothioureyl compounds (Rambacher, cited by Michaud, 1979). Thus, through direct chemical reaction the SH-groups of important enzymes (f.i. catalase), Co-enzymes (f.i. CoA) and redox systems, such as cysteine ↔ cystine and glutathione GSH ↔ GSS are completely blocked and electrolyte leakage is increased (Fuchigami and Nee, 1987). In Cercosporella extract, with 2.5×10^{-2} M cyanamide a 50% inhibition was acquired already after 30 min of exposure (Amberger, 1964a).

In the early experiments of Hofmann et al. (1954a), barley seedlings fed with cyanamide showed a high content of the amino acids alanine and arginine and to a lesser degree also aspartate, demonstrating a rapid

incorporation of cyanamide into amino acid metabolism. In further experiments already after a 6 h chase with cyanamide increased alanine, leucine, valine, glutamate and γ-aminobutyrate contents could be determined semiquantitatively (Table 1). According to Schlegel (1981) and Richter (1982), glutamate and alanine are primary amino acids, built through direct reductive animation. Alanine can also be the result of amino transferases (Taiz and Zeiger, 1991). Leucine and valine are formed from alaline, γ–aminobutyrate, a nonprotein amino acid, is decarboxylated from glutamate and functions as a donor of the NH_2-group. Other amino acids are formed through transamination.

The combined enzymes cyanamide hydratase and urease degrade cyanamide rapidly to NH_3 and CO_2. The highly toxic ammonia will be protonated to the lesser toxic NH_4, and assimilated via the GOGAT pathway (Hess, 1991) to glutamine and glutamate. This detoxification process runs quickly. A preceding carbohydrate catabolism delivers 3 C skeletons (pyruvate) and 5 C skeletons (ketoglutarate) for the amino acid synthesis. In experiments with spinach, barley and rape seedlings after 7 h feeding with cyanamide an enhanced hydrolysis of total and reducing sugars appeared in shoots and roots (Table 2) and increased respiration rate (Table 7).

With continual high NH_3 supply from cyanamide decomposition amides (glutamine and asparagine), the transport and storage forms of nitrogen and basic amino acids are formed such as histidine and lysine (all with 2 N atoms) and mainly arginine (with 4 N atoms and the guanidino group in δ-position) providing the rapid detoxification of ammonia.

In experiments with maize, cabbage, sunflower and rape seedlings, the arginine content of the cyanamide group was some hundred percent higher than that of the nitrate control (Table 3). Also, urea-fed plants (not noticed here) showed an increase (69%) from the urea hydrolysis whereas the ammonium fed plants remained below the control (Wünsch and Amberger, 1974). Rape seedlings fed with cyanamide and exogenically applied ornithine reacted with a 20-fold enhancement of arginine in the roots where the ammonium detoxification mainly takes place (Table 3) (Wünsch and Amberger, 1989).

Table 2. Total sugar and reducing sugar content (mg/100 g dr. m.) of cyanamide fed spinach, barley and rape seedlings.

Cyanamide	Shoots				Roots			
(25 mg N/l)	Total sugar		Reducing sugar		Total sugar		Reducing sugar	
Exposure time (h)	Control	Cy	Control	Cy	Control	Cy	Control	Cy
Spinach								
7	3785	2828	405	340	1153	1001	542	414
31	4394	4220	348	318	1029	957	372	320
Barley								
7	1409	1235	593	545	530	452	327	252
31	1413	1065	571	528	531	444	405	313
Rape								
7	2893	2370	2453	2036	1207	968	879	748
31	2501	1740	2035	1288	947	849	748	713

Cy, Cyanamide; Control, $Ca(NO_3)_2$.

Table 3. Effect of cyanamide nutrition (100 mg N/l nutr. solution) on arginine, lysine, histidine and γ-aminobutyrate content (µM/1 g dr. m.) of plant seedlings.

	Arginine			Lysine		Histidine		γ-aminobutyrate	
Plants	Control	Cy	Cy + ornithine	Control	Cy	Control	Cy	Control	Cy
Cabbage leaves	3.46	4.84							
Maize leaves	1.56	4.59							
Sunflower leaves	2.27	6.81							
Rape leaves	0.80	6.60	12.2	1.2	8.6	Traces	5.4	8.6	3.0
Rape roots	1.50	1.50	33.4	2.5	4.5	1.0	2.0	66.3	33.6

Cy, Cyanamide; Control, $Ca(NO_3)_2$.

Table 4. Total-N and protein-N of maize seedlings.

	Total-N	Protein-N
100 mg N/l. nutr. sol.	mg/100 g dr. m.	
$Ca(NO_3)_2$	306.3	258.2
Cyanamide	396.5	285.0

Also, the lysine and histidine titer of rape leaves and roots was dramatically enhanced by cyanamide feeding obviously at the expense of (decreasing) γ-aminobutyrate. In grapevine seedlings fed with $H_2C^{15}N_2$ for 10 days two thirds of the assimilated total N and about 7% of free arginine were labelled with ^{15}N (Vilsmeier and Amberger, 1988). Thus, it is not surprising when maize seedlings after 18 h cyanamide exposure showed a dramatic increase of total N (29%) and protein-N (10%) compared to the control (Table 4).

In this context, it may be of interest that also a direct chemical arginine synthesis from ornithine and cyanamide is long enough known in Organic Chemistry (Holleman and Richter, 1951) forming guanidine (see formula).

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    COOH                                    COOH
H₂N – CH    + HN = C = NH             HC – NH₂
    CH₂                                     CH₂
    CH₂                                     CH₂ NH₂
    H₂C – NH₂                           H₂C – N  C = NH
                                               H
   Ornithine        Cyanamide           Arginine
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The enzymatic synthesis of arginine follows from

Table 5. Effect of short-time cyanamide treatment of cereal seedlings (g dry m/6 plants).

75 mg N/l nutr. sol.	Wheat		Rye	
	Shoots	Roots	Shoots	Roots
Ca(NO$_3$)$_2$	2.02 ± 0.15	0.99 ± 0.07	3.21 ± 0.02	0.74 ± 0.04
	=100	=100	=100	=100
Interrupted with cyanamide				
For 3 h	168	165	142	166
6 h	173	131	110	131
12 h	162	132	87	81

glutamate via ornithine and citrulline. For remobilization and recycling of arginine into nitrogen metabolism, there are two ways (Bagni, 1986) either with arginase activity to ornithine and urea – that is the case during seed germination – or through decarboxylation to agmatine and CO$_2$ (Nultsch, 1986) and other amines. However, as the arginase activity is strongly inhibited by cyanamide (Wünsch and Amberger, 1989), the pathway is open to polyamines. Arginine decarboxylase is a general stress enzyme. The basic amino acids function as nitrogen pool and are the precursors of polyamines, a new class of growth regulators that are mainly built under stress conditions.

In field experiments, very often a striking stimulation of plant growth can be noticed after Ca-cyanamide ("Kalkstickstoff") application demonstrating an intensive green colour and bushy habit. To clear up this phenomenon, cereal seedlings were grown in nutrient solution with Ca(NO$_3$)$_2$, later on one group was fed with cyanamide in equal N-concentration for 3, 6 and 12 h and then retransferred again to nitrate. After some days, significant growth stimulation was observed in shoots and roots of wheat and rye (Table 5). Plants exhibit a different sensitivity to cyanamide; however, more important is the development stage of the plants. Young seedlings were not able to detoxify high ammonia supply from cyanamide degradation because they are poor in carbohydrates and are not able to provide enough skeletons for amino acid synthesis. However, well developed plants answer with increase of amides and basic amino acids (Wünsch and Amberger, 1974), the precursors of polyamines.

These growth regulators induce cell proliferation, protein synthesis, flowering, fruit initiation and other processes (Slocum and Flores, 1991). In the experiments of Gerandàs (1992) and Gerandàs and Sattelmacher (1995), maize seedlings fed with ammonium answered with a dramatic enhancement of putrescine and spermidine in shoots and particularly roots. According to Marschner (1986), ammonium nutrition activates the arginine mediated pathway followed with a high increase of polyamines. Bagni (1986) saw the growth stimulation on cellular level in parallel with the increase of polyamines.

Thus, the often observed growth stimulation after Ca-cyanamide application could possibly be the effect of polyamines. Admitted, this may be speculative and needs to be verified by further investigations.

Cyanamide induced enzyme reactions and respiration

The most dramatic physiological effect of cyanamide is the strong inhibition of catalase (Amberger, 1961a, b) caused by the reaction of the nitrile group with thiols and haematin of the enzyme. With a crystallized enzyme preparate (from bovine liver), the catalase activity was inhibited to 50% by a cyanamide concentration of 5×10^{-2} M. This inhibition is reversible as far as a sublethal dosis is used (Amberger, 1964a, b). Thaler (1990) succeeded a 50% inhibition already with 2 mM and a 80% inhibition with 4 mM cyanamide using a more sensitive method (O$_2$-electrode). These results, originally acquired with a pure enzyme could be confirmed in many plant experiments with crude enzyme preparates.

In sugar beets fed with 25 mg N/l nutrient solution (~ 10$^-$3 M) cyanamide and 6 h exposure time the catalase activity in shoots decreased to 72% compared to the nitrate control and remained on 25% level for the rest of exposure time (Amberger and Wünsch, 1963). Roots reacted much more sensitive, already after 3 h with 80% and finally with 36% activity (Table 6). Plants with high catalase activity (mainly dicotyledons) react more severely on cyanamide compared to monocotyledons.

The function of catalase in plant metabolism is to control the H$_2$O$_2$-concentration, the product of photosynthetic electron transport and the end product of flavoprotein enzyme activities. By scavenging oxidative radicals, catalase protects the redox systems from intoxication and irreparable damages.

The peroxidase activity in horse radish and also in the sugar beet experiment (Table 6) is initially more or less unchanged in shoots and roots. However, after 12 h cyanamide exposure and the beginning of wilting the activity increased up to 43% as the result of chlorophyll and protein breakdown. In an experiment with rape seedlings and higher cyanamide (50 mg N) supply, the peroxidise activity is first associated with a great

Table 6. Effect of cyanamide on catalase and peroxidase activity of sugar beet seedlings (activity/100 mg dr. m).

25 mg N/l nutr. sol.	Exposure time (h)				
	3	6	9	12	36
Catalase activity					
Shoots					
Control	100	100	100	100	100
Cyanamide	99	72	38	21	27
Roots					
Control	100	100	100	100	100
Cyanamide	80	55	42	38	36
Peroxidase activity					
Shoots					
Control	100	100	100	100	100
Cyanamide	101	99	109	114	143
Roots					
Control	100	100	100	100	100
Cyanamide	107	112	111	105	140

Control, $Ca(NO_3)_2$.

Table 7. Effect of cyanamide on peroxidase activity and respiration of rape seedlings.

50 mg N/l nutr. sol.	Exposure time (h)			
	Mild wilting		Severe wilting	
	4	28	52	76
Peroxidase activity/100 g dr. m.				
Shoots				
Control	86.7 = 100	99.4 = 100	56.9 =100	60.7 = 100
Cyanamide	113	110	140	148
Roots				
Control	282.6 = 100	197.4 = 100	147.8 = 100	142.0 = 100
Cyanamide	98	132	168	183
Respiration $\mu l O_2$/100 mg dr. m.				
Shoots				
Control	12.0 = 100	11.3 =100	---	21.1 = 100
Cyanamide	143	196	---	145
Roots				
Control	42.5 = 100	76.8 = 100	67.0 = 100	62.4 = 100
Cyanamide	155	140	121	108

Control, $Ca(NO_3)_2$.

enhancement of respiration (O_2-uptake) and is later followed with sharp decrease and irreparable damages (Table 7). The peroxidase enzyme utilizes different organic electron donors for the reduction of H_2O_2 and
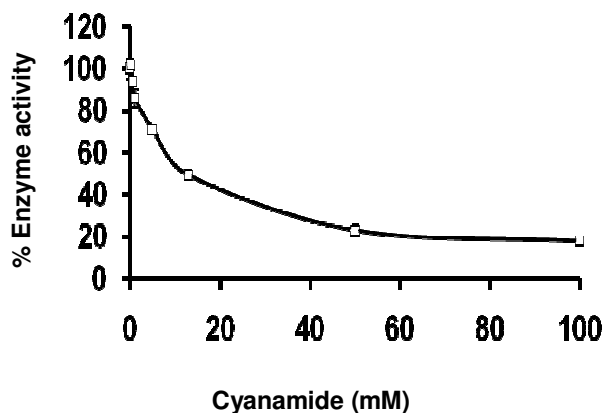
Figure 1. Effect of cyanamide on cytochrome$_c$-oxidase activity (not inhibited cytrochrome$_c$-oxidase activity = 100).

Table 8. Effect of cyanamide application (3% Dormex) on flower bud break of apple and pear varieties in the growing season 1992 and 1993.

| Parameter | Flower buds | | | |
| | 50% open | | Fully open | |
	1992	1993	1992	1993
Anna apple	01 March	03 March	01 April	25 March
Control cyanamide	16 February	04 February	21 March	10 February
Einshamer apple	21 March	25 March	20 April	22 April
Control cyanamide	28 February	01 March	28 March	09 March
Lecont pear	20 March	25 March	10 April	12 April
Control cyanamide	15 March	12 March	28 March	28 March

Cyanamide treatment: Apple, 17 December; Pear, 03 January.

lignin biosyntheses.

Cyanamide proved also to be a very strong inhibitor of cytochromoxidase. The pure enzyme (from bovine heart) was inhibited to 50% with 10 mM and to 80% with 50 mM cyanamide (Figure 1) (Thaler, 1990). Pre-treatment of the enzyme with cyanamide enhanced the inhibition. In a chloroplast suspension from sunflower leaves, the cytochromoxidase was inhibited to 60% with 10^{-3}M cyanamide (not noted here).

Also, the copper protein ascorbicacid oxidase, isolated from pumpkin, which controls the system ascorbic acid ↔ dehydroascorbic acid was inhibited to 50% with cyanamide in a concentration of 5×10^{-2}M (Amberger

and El-Fouly, 1964). This enzyme is involved mainly in the germinating process and early growth of plants. When the normal energetic pathway via the cytochrome system is blocked, then a relatively inefficient energy releasing CN-insensitive – and obviously also cyanamide insensitive – respiration takes place, stimulated with the H_2O_2-accumulation as a result of inhibited catalase.

Cyanamide breaking dormancy

Dormancy is the natural rest phase of fruit trees. In many temperate and subtropical areas, mild winter temperatures are not low and long enough to break the dormancy (< 7°C for 100 to 150 h) to satisfy the cold-requiring temperatures of fruit trees, such as apples, pears, peaches, grapevines etc. followed with retarded and irregular onset of flower buds and consequently with lower yields and fruit quality.

Japanese scientists (Kuroi et al., 1963) have first reported about experiments with cyanamide as the most effective agent to induce earlier and uniform bud break. In extensive experiments, Shulman et al. (1983, 1986) sprayed a hydrogen cyanamide solution (Tradename „Dormex", SKW-Trostberg) in a concentration of 1 to 3% on the dormant branches of fruit trees in early spring with convincing results. There are now numerous publications available from many countries where these methods are applied regularly.

In an experiment at El-Fayoum Research farm carried out by Seif El-Yazal (1997) in the seasons 1992 and 1993, Anna apple trees treated with 3% cyanamide solution reached 50% bud opening 13 to 27 days earlier and fully blooming 11 to 44 days earlier than the water sprayed controls. The corresponding data of the Einshamer variety ranged between 21 to 24 days, respectively 23 and 44 days of advancement. The pears reacted on cyanamide treatment with 5, respectively 15 days earlier blooming (Table 8). These data are in agreement with the results of Hasseb and Elezaby (1995) at Giza Station Egypt. The results depend on the cyanamide concentration (1 to 4%) optimal for the different crop and the best timing.

In our experiments with grapevine seedlings, a maximum effect of bud break (Figure 2) was acquired with 200 mM cyanamide (Thaler, 1990). In turions of Elodea densa, 18 h fed with 15 mM cyanamide increasing respiration was noticed (Figure 3) (Thaler, 1990) as a consequence of catalase and cytochromoxidase inhibition.

Cyanamide solution penetrates easily into the bark and induces hydrolysis of storage protein and carbohydrates. Arginine is the dominant constituent of bark protein and comprises about 75% of the soluble nitrogen fraction (Tromp, 1970). Prior to the germination of flower buds, arginine is recycled in nitrogen metabolism. With inhibited arginase activity (Wünsch and Amberger, 1989), the
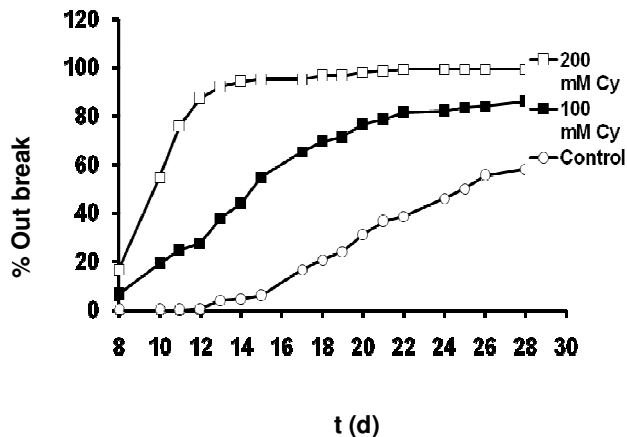
Figure 2. Effect of cyanamide (Cy) application on flower bud break of grapevine seedlings.
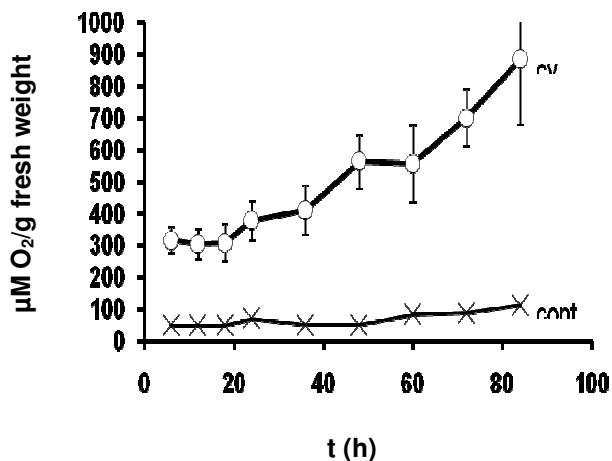

Figure 3. Alternative respiration of turions after 18 h application with 15 mM cyanamide.

Table 9. Effect of cyanamide application on arginine (mg/100 g dry weight) and polyamine content (mg/kg dry weight) of Anna apple flower buds (1993).

	Bud development			
Polyamines	Dormant (03 January)	Swelling (21 January)	50% open buds (07 February)	Full blooming (17 February)
Arginine	479	-	324	188
Putrescine	34	46	86 (36)	62
Spermidine	34	66	83 (25)	58
Spermine	290	142	606 (207)	372
Histamine	51	31	221 (12)	0
Cadaverine	996	2033	4122 (5182)	5804

In brackets, dates of untreated control.

decomposition followed by arginine- and ornithine decarboxylases to polyamines (Bagin, 1986). The hydrolysis of bark protein coincides with a sharp rise of polyamines prior to bud opening of apple trees (Wang and Faust, 1993; Costa and Bagni, 1983).

Polyamines originating from basic amino acids such as arginine, ornithine, histidine, lysine etc. are decarboxylated to putrescine, spermidine, spermine, histamine and cadaverine. These basic compounds play an essential role in cell division and morphogenesis, flowering and fruitset. A very comprehensive study is made by Slocum and Flores (1991). Artificial chilling as well as the application of chemicals, such as cyanamide favours this decarboxylation process.

In our experiments, the Anna apples (Table 9) disposed a sharp decrease of the high arginine content of the dormant flower buds to the fully blooming stage corresponding with a rapid increase of the polyamine content from the low level of dormancy up to maximum when 50% buds were open. Later on follows a sharp decrease down to the blooming stage. Cadaverine is the only amine that showed a nearly 6-fold increase from dormancy to fully blooming; also the untreated control was high when 50% of buds were open. The analysis was kindly done by Henle et al. (1991) and Krause et al. (1995).

Also, Bissi et al. (1988) noticed in swelling fruitlets of apples high polyamine levels associated with rapidly growing cells and later on a sharp decrease of polyamines. Similar results in apple flowers are reported by Edwards (1986). Mosbach et al. (1990) noticed a 50 and 40% increase of putrescine and spermine from dormant to early opening of citrus flower buds.

In cherry flower buds, the concentration of putrescine, spermidine and spermine increased 10 to 20 fold from dormancy to initial swelling and decreased again to fully blooming (Wang, 1985). In *Helianthus tuherosus* tubers noticed D'Orazi and Bagni (1987) already 1 h after dormancy break a 10-fold increase of arginine and ornithine decarboxylases and rapid increase of putrescine, spermidine and spermine and also DNA and RNA content. Flower bud induction and tuberization seem to be linked. Similar results are reported by Heimer et al. (1979) in early stage of tomato growth.

Kaur-Sawhney et al. (1982) noticed an immediate decrease of stored arginine and an increase of polyamines in *H. tuberosus* tubers, Northcot and Nowak (1988) in seed potatoes after dormancy break.

The enzyme catalase plays an important role in bud germination. In dormant buds, catalase activity is maximum and decreases with low winter temperature or artificial chilling (Nir et al., 1986). Grapevine buds treated with 0.25 M cyanamide caused a 100% bud break, catalase activity reduced to 50% and H_2O_2-level increased (15%) in bud tissue (Nir, 1993). In an additional experiment, the application of 30 mM H_2O_2 increased significantly the bud opening. In cyanamide treated

turions, the higher H_2O_2 level increased the GSSG rate in the redoxsystem (Thaler, 1990). Fuchigami and Nee (1987) saw the disulfide form of glutathione closely involved in the rest breaking action of cyanamide. Application of H_2O_2 as well as catalase inhibition (with thiourea) and also genetic repression of catalase increased sprouting of potato tubers (Bajji et al., 2009).

These results confirmed the proposal of Hendricks and Taylorson (1975) and Roberts and Smith (1977) that the catalase inhibition by cyanamide is the key reaction and the followed H_2O_2 overflow causes the oxidation of nicotinamide adenine dinucleotide (NADH) to NAD^+ which is the essential coenzyme of glucose-6-dehydrogenase initiating the oxidative pentose phosphate pathway (PPP) of glucose use that is typical for fruitset and early growth (Hu and Couvillon, 1990). A direct evidence is delivered through determination of the C_6/C_1-ratio of the glucose break, that decreased in cyanamide treated turions after 6 h incubation with 15 mM cyanamide from 0.97 (control) to 0.67 (Thaler, 1990), similar to the chilling treated ones. In fully opened buds the oxidative PPP turns over again to the reductive Calvin Cycle.

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