Cyanamide in plant metabolism

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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 thiol s of redox systems. Cyanamide applied to dormant buds of fruit trees inhibits catalase activity and increases H$_2$O$_2$-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.

\[ N \equiv C-NH_2 \leftrightarrow HN = C = NH \]

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). Lamarre and Brunel (1951) reported that the fungus Sterigmatocystis nigra is able to hydrolyze 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) Radioautography ($^14$CN$_2$) (Hofmann and Latzko 1954; Amberger and Latzko 1954)
2) Emissions spectroscopy ($^15$C$_2$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)
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 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$_2$C$^{15}$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.

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. Japanese researchers, Kamo et al. (2003, 2006a, b) succeeded to verify the biosynthesis of cyanamide de novo in two Vicia species (villosa and cracca) acting as

| 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 the buds of Elodea densa water lens. Additional experiments were also carried out with turions, the rest samples were dispersed in 10 ml of 0.1 mol/l hydrochloric acid, supernatant was used for chromatographic analysis. Polyamines concentration of 20% and centrifugation at 5000 g for 15 min. The 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.

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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 no-tillage 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 dependent 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 H₂O₂-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 NH₂-group. Other amino acids are formed through transamination.

The combined enzymes cyanamide hydratase and urease degrade cyanamide rapidly to NH₃ and CO₂. The highly toxic ammonia will be protonated to the lesser toxic NH₄⁺ 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 NH₃ 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 1. Amino acid content of barley seedlings fed with cyanamide (50 mg N/l nutr. solution).

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cy</td>
<td>Control</td>
<td>Cy</td>
<td>Control</td>
</tr>
<tr>
<td>Alanine</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Leucine</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Valine</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Glutamate</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>γ-aminobutyrate</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

+, Low; ++, higher; Control, Ca(NO₃)₂; Cy, cyanamide.
Table 2. Total sugar and reducing sugar content (mg/100 g dr. m.) of cyanamide fed spinach, barley and rape seedlings.

<table>
<thead>
<tr>
<th>Cyanamide (25 mg N/l)</th>
<th>Shoots</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total sugar</td>
<td>Reducing sugar</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Cy</td>
</tr>
<tr>
<td>Spinach 7 exposure</td>
<td>3785</td>
<td>2828</td>
</tr>
<tr>
<td>Spinach 31 exposure</td>
<td>4394</td>
<td>4220</td>
</tr>
<tr>
<td>Barley 7 exposure</td>
<td>1409</td>
<td>1235</td>
</tr>
<tr>
<td>Barley 31 exposure</td>
<td>1413</td>
<td>1065</td>
</tr>
<tr>
<td>Rape 7 exposure</td>
<td>2893</td>
<td>2370</td>
</tr>
<tr>
<td>Rape 31 exposure</td>
<td>2501</td>
<td>1740</td>
</tr>
</tbody>
</table>

Cy, Cyanamide; Control, Ca(NO₃)₂.

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

<table>
<thead>
<tr>
<th>Plants</th>
<th>Arginine</th>
<th>Lysine</th>
<th>Histidine</th>
<th>γ-aminobutyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Cy</td>
<td>Cy + ornithine</td>
<td>Control Cy</td>
<td>Control Cy</td>
</tr>
<tr>
<td>Cabbage leaves</td>
<td>3.46 4.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize leaves</td>
<td>1.56 4.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunflower leaves</td>
<td>2.27 6.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rape leaves</td>
<td>0.80 6.60</td>
<td>12.2</td>
<td>1.2 8.6</td>
<td>Traces 5.4</td>
</tr>
<tr>
<td>Rape roots</td>
<td>1.50 1.50</td>
<td>33.4</td>
<td>2.5 4.5</td>
<td>1.0 2.0</td>
</tr>
</tbody>
</table>

Cy, Cyanamide; Control, Ca(NO₃)₂.

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

<table>
<thead>
<tr>
<th></th>
<th>Total-N</th>
<th>Protein-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg N/l nutrit. sol.</td>
<td>mg/100 g dr. m.</td>
<td></td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>306.3</td>
<td>258.2</td>
</tr>
<tr>
<td>Cyanamide</td>
<td>396.5</td>
<td>285.0</td>
</tr>
</tbody>
</table>

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 H₂C¹⁵N₂ for 10 days two thirds of the assimilated total N and about 7% of free arginine were labelled with ¹⁵N (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).

\[
\begin{align*}
\text{COOH} & \quad \text{COOH} \\
\text{H}_2\text{N} – \text{CH} & + \text{HN} = \text{C} = \text{NH} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{CH}_2 \text{NH}_2 \\
\text{H}_2\text{C} – \text{NH}_2 & \quad \text{H}_2\text{C} – \text{N} \quad \text{C} = \text{NH} \\
\text{Ornithine} & \quad \text{Cyanamide} \quad \text{Arginine}
\end{align*}
\]

The enzymatic synthesis of arginine follows from
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 CO₂ (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(NO₃)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 Gerandás (1992) and Gerandás 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
(O₂-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 H₂O₂-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 5. Effect of short-time cyanamide treatment of cereal seedlings (g dry m/6 plants).

<table>
<thead>
<tr>
<th>75 mg N/l nutr. sol.</th>
<th>Wheat</th>
<th>Rye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(NO₃)₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interrupted with cyanamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For 3 h</td>
<td>168</td>
<td>165</td>
</tr>
<tr>
<td>6 h</td>
<td>173</td>
<td>131</td>
</tr>
<tr>
<td>12 h</td>
<td>162</td>
<td>132</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Ca(NO₃)₂</td>
<td>2.02 ± 0.15</td>
<td>0.99 ± 0.07</td>
<td>3.21 ± 0.02</td>
</tr>
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<tr>
<td>12 h</td>
<td>162</td>
<td>132</td>
</tr>
</tbody>
</table>
Table 6. Effect of cyanamide on catalase and peroxidase activity of sugar beet seedlings (activity/100 mg dr. m).

<table>
<thead>
<tr>
<th>25 mg N/l nutr. sol.</th>
<th>Exposure time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td><strong>Catalase activity</strong></td>
<td></td>
</tr>
<tr>
<td>Shoots</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Cyanamide</td>
<td>99</td>
</tr>
<tr>
<td>Roots</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Cyanamide</td>
<td>80</td>
</tr>
</tbody>
</table>

| **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(NO₃)₂.

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

<table>
<thead>
<tr>
<th>50 mg N/l nutr. sol.</th>
<th>Exposure time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild wilting</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td><strong>Peroxidase activity/100 g dr. m.</strong></td>
<td></td>
</tr>
<tr>
<td>Shoots</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>86.7 = 100</td>
</tr>
<tr>
<td>Cyanamide</td>
<td>113</td>
</tr>
<tr>
<td>Roots</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>282.6 = 100</td>
</tr>
<tr>
<td>Cyanamide</td>
<td>98</td>
</tr>
</tbody>
</table>

| **Respiration µlO₂/100 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(NO₃)₂.

enhancement of respiration (O₂-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 H₂O₂ and
Cyanamide (mM)

Figure 1. Effect of cyanamide on cytochrome-c-oxidase activity (not inhibited cytochrome-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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Flower buds</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50% open</td>
<td>Fully open</td>
<td></td>
</tr>
<tr>
<td>Anna apple</td>
<td>01 March</td>
<td>03 March</td>
<td>01 April</td>
</tr>
<tr>
<td>Control cyanamide</td>
<td>16 February</td>
<td>04 February</td>
<td>21 March</td>
</tr>
<tr>
<td>Einshamer apple</td>
<td>21 March</td>
<td>25 March</td>
<td>20 April</td>
</tr>
<tr>
<td>Control cyanamide</td>
<td>28 February</td>
<td>01 March</td>
<td>28 March</td>
</tr>
<tr>
<td>Lecont pear</td>
<td>20 March</td>
<td>25 March</td>
<td>10 April</td>
</tr>
<tr>
<td>Control cyanamide</td>
<td>15 March</td>
<td>12 March</td>
<td>28 March</td>
</tr>
</tbody>
</table>

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

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 ascorbic acid 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 H₂O₂-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
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 tuberosus 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.


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$_2$O$_2$-level increased (15%) in bud tissue (Nir, 1993). In an additional experiment, the application of 30 mM H$_2$O$_2$ increased significantly the bud opening. In cyanamide treated

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**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).

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

In brackets, dates of untreated control.
turions, the higher H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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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