Memorandum of Understanding 19071/05/NL/CP

UNIVERSITEIT GENT

FACULTEIT WETENSCHAPPEN

# TECHNICAL NOTE: 89.11

## PLANT STRESS RESPONSE: STATE OF THE ART AND IDENTIFICATION OF CRITICAL POINTS

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| reference/*réference* | Contract number 19787/06/NL/PA |
| issue/*édition* | 1 |
| revision/*révision* | 0 |
| date of issue/*date d'édition* | 31Oct06 |
| status/*état* | Final |
| Document type/*type dedocument* | Technical Note |
| Distribution/*distribution* | |

C O N F I D E N T I A L   D O C U M E N T

# APPROVAL

| Title *titre* | issue *issue* 1 | revision *revision* 0 |
|---|---|---|

| author *auteur* | date *date* 31Oct 06 |
|---|---|

| approved by *approuvé by* | date *date* |
|---|---|

# CHANGE LOG

| reason for change /*raison du changement* | issue/*issue* | revision/*revision* | date/*date* |
|---|---|---|---|
| | | | |

# CHANGE RECORD

Issue: 1 Revision: 0

| reason for change/*raison du changement* | page(s)/*page(s)* | paragraph(s)/*paragraph(s)* |
|---|---|---|
| | | |

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# 1. Introduction

Any excessive deviation of an environmental factor (e.g. growth medium, surrounding air, and light) from optimal values imposes stress on plants, and limits their yield in terms of bio-mass. Besides these less optimal growth conditions (abiotic stress factors), also weeds, pests and pathogens of viral, bacterial and fungal nature cause stress (biotic stress factors), due to competition for external resources, or by parasitizing on the plant's resources. Hence, there are numerous sources of stress that need to be contained in order to obtain stable yields These stresses do not just act by themselves, but typically form complex interactions. In modern agriculture the striving is to reduce stress to a minimum as long the measures are cost effective. In reality however, even in intensively controlled agriculture as practiced in Western Europe, losses are unavoidable (Oerke and Dehne, 2004).

In advanced life support systems the introduction of plants is beneficial to reuse $CO_2$ and produce oxygen and quality food for the crew. The emphasis in these closed loop systems is on total control and efficient recycling of all inputs, rather than, or more so than on cost effectiveness. Control to such extent is new and therefore systems need to be developed that guarantee (nearly) stress-free crop growth. Many of the stresses known from common agriculture will also be relevant for these life-support systems though certain types of stress can be excluded to great extent. Light for instance, will always be an important input factor and less than optimal supply will compromise yield in any system. Weeds, on the other hand, are a large but specific problem for soil-bound cultures, which can be totally avoided by switching to a closed hydroponic system. Besides the known stresses there will also be new stresses specific for closed systems, such as accumulation of volatile compounds (e.g. accumulation of ethylene and other volatile organic components) to threshold-exceeding levels, for which specific measures will need to be implemented. Furthermore, for space applications there will be additional specific stresses related to unique environmental conditions such as lack off or reduced gravity.

In this technical note we will list the potential plant stresses which will need to be dealt with in advanced life support systems. The importance and action radius of these stresses will likely strongly vary from crop to crop. For obvious reasons, we will focus on the MELiSSA crop list, and will present an overview and discuss the stresses as described in literature.

# 2. Environmental parameters

Potential crop yield is defined as maximum crop yield under optimal environmental conditions and absence of weeds, pests and pathogens. Theoretically it has a fixed value for every crop since it is only limited by its genotype. This value can be optimized by plant breeding. However, in general, actual yields are much lower than this potential yield due to sub-optimal conditions (Oerke and Dehne, 2004). In a closed culture system the yield should theoretically approach this potential yield much closer since all environmental factors can be set to a constant optimum. So provided the equipment required for generation of the proper environment keeps running, yields should be predictable and high. The absence of equipment failure can however not be guaranteed and in case of a failure conditions often evolve from optimal to worst, e.g. from optimal light to dark in case of a power/electricity failure. It will be important to establish how long plants can survive under such conditions, and what the consequences on production will be. In addition, possible emergency measures to be taken to reduce damage associated with equipment malfunction are very important to be considered. In case of electricity failure for example it's better to provide plants with a minimal amount of light (eg from an emergency power unit or battery, than to leave them totally in the dark (http://www.usu.edu/cpl/research_failure.htm) (Taiz and Zeiger, 2006).

Different crops and even cultivars are differently adapted to environmental factors and have therefore different optimal values and acceptable ranges for given environmental factors. The most important environmental (abiotic) factors determining crop yield are: incident photosynthetic photon flux (light), concentration of carbon dioxide ($CO_2$) in the air, temperature, availability of water and nutrients.

Abiotic stresses are having –in field conditions- far more impact on crop losses than biotic factors do (Buchanan et al., 2000; Chapter 22). For eight major crops, abiotic losses range between 50 and 82%.

Setting these factors optimally results in a maximal growth curve which is fixed dependent on the genotype. Eckart (1996) has defined five classes of crops based on the maximal growth rate and required conditions to achieve those rates. Water and nutrients are not mentioned in this work but are often assumed to be optimal in alternative life support systems which are based on hydroponic cultures. The MELiSSA crops fall into three different classes. The class with the least maximum growth rate (20-30 g $m^{-2}.day^{-1}$) contains $C_3$ plants including the MELiSSA crops potato and wheat. The second class of $C_3$ plants with a maximum growth rate of 30-40 g $m^{-2}.day^{-1}$ contains the MELiSSA crop soybean. The third class contains $C_4$ plants (30-60 g $m^{-2}.day^{-1}$) including the MELiSSA crop rice. Tomato is classified in class I as well as II, indicating the diversity of genotypes among tomato cultivars. The fourth and fifth classes cover some additional $C_4$ and the CAM plants respectively, which are not relevant for the present choice of crops. Other MELiSSA crops (onion, table beet, lettuce and spinach, which are all $C_3$) were not mentioned in the table by Eckart (1996). Based on the paper from Franz et al. (2004), describing optimal growth conditions for lettuce (in a hydroponic system), lettuce does not seem to fit in any of the above mentioned classes. Its optimal growth temperature varied between 25 and 30 degrees Celsius, which corresponds to class II, but its maximum growth rate was only 19 $g.m^{-2}.day^{-1}$, which corresponds more to class I.

## 2.1. Light

Plants absorb radiation of 400-700 nm in chlorophyll containing cells and use it as energy for photosynthesis (to transform $CO_2$ into sugars). Therefore radiation between 400-700nm is called PAR (Photosynthetic Active Radiation). Solar radiation contains not only PAR but also a fraction UV radiation (300-400nm) and infra-red radiation or heat (>700nm).

### 2.1.1.Photoperiod

Some plants need a defined photoperiod (daylength) to complete their life cycle. This is especially important for completion of the life cycle, i.e. for the conversion from a vegetative to a reproductive state. Some plants require a minimum number of days where the uninterrupted dark period exceeds a minimum duration, for floral evocation. These are called short-day (SD) plants. Short Day Plants (long night plants), typically initiate reproductive behavior when daylength drops below 12 hours. Long-day (LD) plants, on the other hand, flower only when the photoperiod exceeds a certain value for a minimum number of cycles (ie days). Long day plants (short night plants) typically initiate reproductive behavior when daylength exceeds 12 hours. Species that do not require a certain daylength to flower are called day-neutral. Some plants have an absolute or obligate photoperiodic requirement to flower (qualitative photoperiodic response), while others will flower faster in appropriate photoperiods, but will ultimately flower even under unfavorable daylength conditions (quantitative photoperiodic plants) (Jones, 1992). For the current MEliSSA crops the photoperiodic requirements are as follows:

| | |
|---|---|
| obligate SD plants: | *Glycine max* (soybean) (Jones, 1992) |
| quantitative SD plants: | *Oryza sativa* (rice) (Jones, 1992) |
| obligate LD plants: | *Spinacia oleracea* (spinach) (Jones, 1992) |
| quantitative LD plants: | *Beta vulgaris* (beet) (Jones, 1992) |
| | *Triticum aestivum* (wheat) (Jones, 1992) |
| day-neutral plants: | *Lycopersicum esculentum* (tomato) |

Many modern varieties, e.g. of soybean, wheat and rice, are less rigorously controlled by daylight than are related wild species due to breeding (Jones, 1992). Therefore it is advisable to check the daylength requirement for individual varieties.

From origin lettuce (*Lactuca sativa*) is a long-day plant (http://aggie-horticulture.tamu.edu/syllabi/325/schedule/Crops/Leafy/Leafy%20Crops%20Handout%206%20per%20page.pdf#search='photoperiod%20and%20lettuce'), however many modern varieties are day-neutral.

For onions (*Allium cepa*) daylength is important for bulb development. Onion varieties can have different requirements regarding the number of daylight hours required to initiate bulb formation. There are long-day onions which set bulbs when receiving 15 to 16 hours of daylight and there are short-day varieties, which set bulbs with about 12 hours of daylight. (http://vric.ucdavis.edu/veginfo/commodity/garden/crops/onion.pdf)

While many wild species of potato are obligate short-day plants (Amador et al., 2001), the majority of bred potato cultivars are not day length sensitive (http://www.uga.edu/vegetable/potato.html).

For some crops or varieties, e.g. soybean, the photoperiodic requirement will need to be fulfilled to initiate flowering and consequently to be able to harvest. If the day length is not important for the development a long day to maximize radiation interception and hence, photosynthetic yield, is preferred. Many lettuce varieties for example are day-neutral and longer-days will reduce the crop cycle time and enhance the yield. In combination with high temperatures however some lettuce varieties might still respond with early flowering, rendering the lettuce unsuitable for consumption.

### 2.1.2.Quality

The spectrum of the used light source has a minor impact on photosynthetic efficiency. However, spectral composition can have profound effects on plant development and morphology (ref) (Sager and McFarlane 1997). In view of the choice of illumination type, experiments with LED powered growth of lettuce gave some indications on the optimal combination of LED types to use (Brazaityte et al., 2006; Kim et al., 2004)

### 2.1.3.Intensity

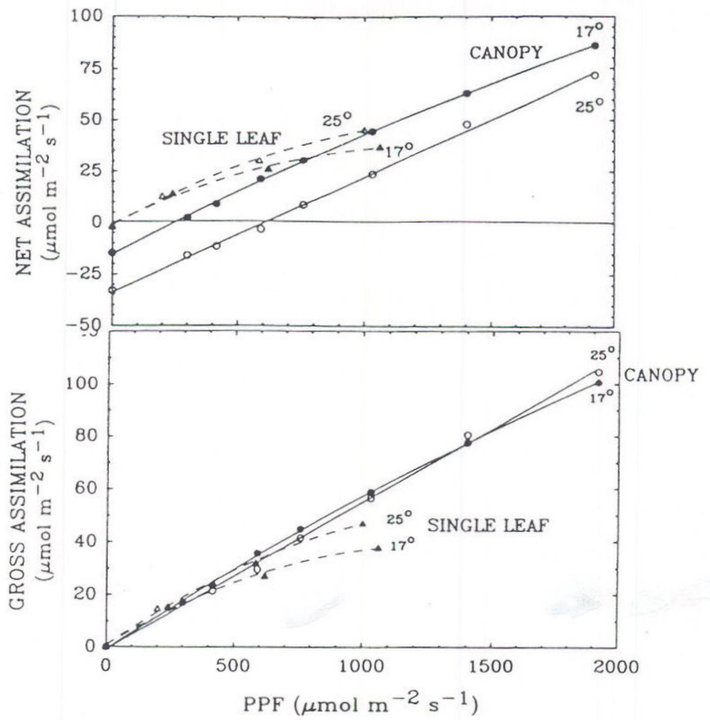


**Figure 1:** The photosynthetic response of individual wheat leaves and of the intact wheat canopy. The leaves light-saturate at a PPF (photosynthetic photon flux) of about 1000 µmol $m^{-2}$ $s^{-1}$, but canopy photosynthetic rate is linear, even up to the equivalent of full sunlight (2000 µmol $m^{-2}$ $s^{-1}$). The canopy was grown at a constant 21° C with elevated $CO_2$ (1200 µmol $m^{-2}$ $s^{-1}$). The photosynthetic rate of the single leaves is expressed on a leaf-surface-area basis, and the canopy photosynthetic rate is expressed on a horizontal-surface-area basis. The leaf area index (LAI) of the canopy exceeded 10, which results in a high dark respiration rate, a high light compensation point, and a linear response to increasing PPF (From Bugbee,1994).

For spinach the potential light saturation point based on fresh weight was determined to be 775 µmol $m^{-2}$ $s^{-1}$ in a hydroponic system (Lefsrud *et al*., 2006).

Since light is crucial for plant growth, and power failures leading to darkness cannot be excluded it is important to know how plants can recover from such periods and how recovery can be enhanced. In this respect the Crop Physiology Laboratory headed by Bruce Bugbee (Utah State University) has done some interesting studies to show that the effects of a long period of darkness can be mitigated by lower temperature and low light conditions (http://www.usu.edu/cpl/research_failure.htm). Power failures with shorter times of optimal light quantity availability will likely benefit from applying these measures.

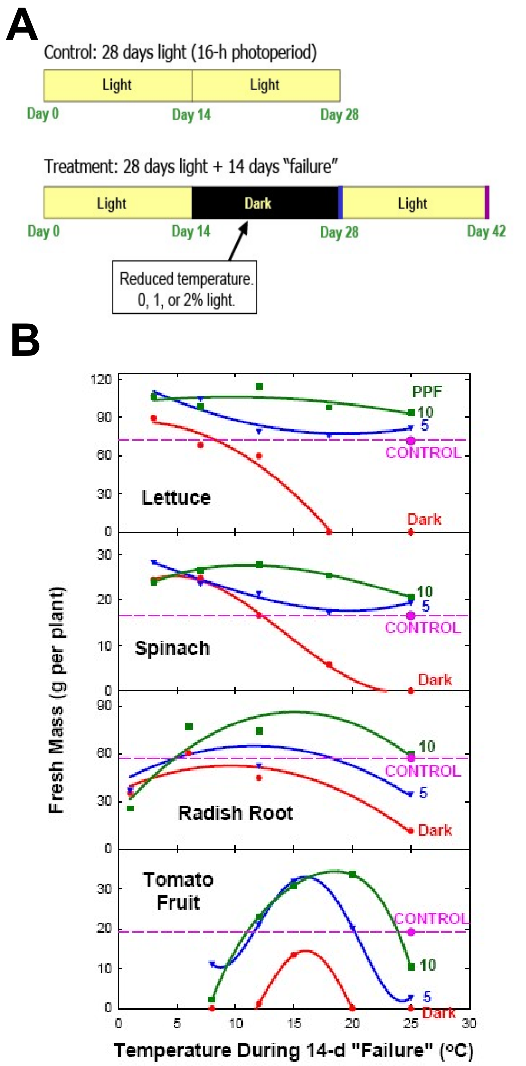


**Figure 2:** Recovery from a 14-day dark period

## 2.2. $CO_2$

$CO_2$ is essential for photosynthesis and thus plant growth. Current atmospheric $CO_2$ levels on earth are about 350 ppm. For many crop plants at high light intensities this is not the optimum. This can be seen in figure 3, which shows the net photosynthetic rate for potato stands in response to $CO_2$. This depicts a classical example of a $C_3$ plant response to $CO_2$. Rates increased rapidly as $CO_2$ was increased up to about 500 ppm, and saturated near 1000 ppm. The minimum amount of $CO_2$ required in this example to start net photosynthesis (the so called $CO_2$ compensation point) is about 80 ppm. These values for net photosynthesis ($CO_2$ exchange rates) will vary with crop age, crop species and in combination with other environmental factors like temperature. This example tells us that photosynthesis and thus crop yield can be increased by enhancing $CO_2$ levels above ambient. However it also includes a warning against $CO_2$ levels dropping below ambient levels, causing large productivity losses. From horticulture it is known that $CO_2$ levels below ambient are not uncommon. Especially on cold but sunny days, when windows in greenhouses close, levels of $CO_2$ drop and even though radiation is high photosynthesis will drop. It is also common practice in greenhouses for e.g. tomato production to increase $CO_2$ to 1000 ppm. Enhancing levels till 1500 ppm is only done in tomato production in greenhouses if a cheap source of $CO_2$ is available, since otherwise less enhanced yields do not weigh up to the extra costs. Even higher levels of $CO_2$ are not expected to be toxic for plant or to reduce yield, but may also not lead to desired yield increases (personal communication, Belgium Research Center Hoogstraten) (Taiz and Zeiger, 2006).

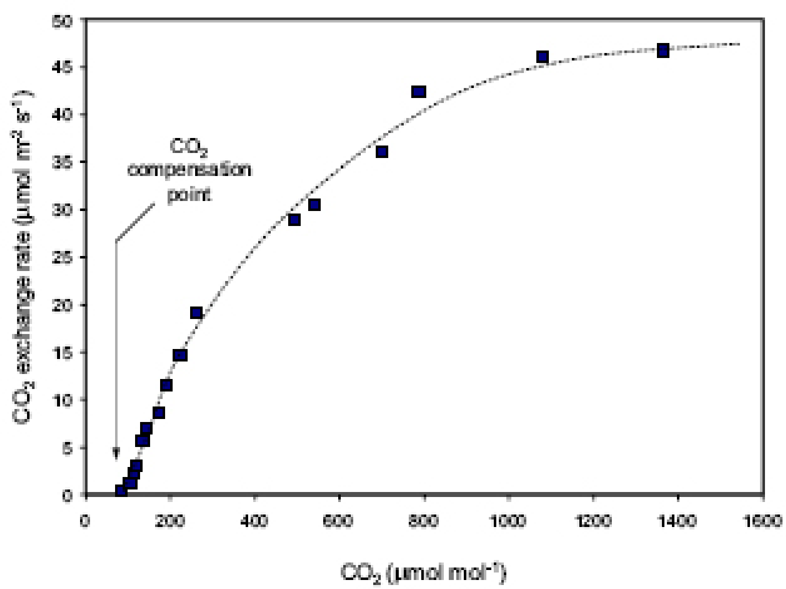


**Figure 3**: Effect of $CO_2$ concentration on $CO_2$ exchange (net photosynthesis) rates for 20 $m^2$ stand of potato (cv. Norland). Note that potato shows a classic C3 response, with photosynthetic rates saturating near 1000 µmol $mol^{-1}$. CO2 compensation point for the stand was near 80 µmol $mol^{-1}$. (From Wheeler, 2006)

## 2.3. Temperature

Most plants can only grow over a limited range of temperatures from somewhat above freezing point to around 40 degrees Celsius, while growth approaches the maximum over an even more restricted temperature range that depends on species and growth stage (Jones, 1992). The latter point is illustrated by spinach yield in relation to temperature as determined by Lefsrud et al. (2006). In a hydroponic system the maximum fresh weight was achieved at 20 °C. Five degrees below or above the optimum temperature the yield was reduced to 77%, while with an additional five degrees lower temperature the yield dropped to 21% of the maximum yield.
During the light period, if provided with ample light, the rate of photosynthesis exceeds the rate of respiration and net growth occurs. At night only respiration continues and stored energy reduces (slowly). Cooler nights are applied to reduce the rate of respiration and thus reduce losses during the dark period, although the effectiveness of this approach might be questioned.
To achieve an optimal yield the temperature for photosynthesis should be optimal. However for plants which need to flower the temperature during anthesis might need to be controlled within tight limits.

## 2.4. Water

Water should be constantly available to the plant via the roots. Roots of most plants can be grown in a hydroponic solution without restrictions, provided soluble oxygen levels are maintained as high as possible (Yoshida and Eguchi, 1994). To guarantee optimal oxygen supply to the root an aeroponics system might be favorable (Kratsch et al., 2006). In this respect NFT (nutrient film technique) or aggregate (using an inert granular substrate) hydroponics also might perform better than deep water hydroponics. In a weightless environment the nutrient solution supply becomes even more challenging due to diffusion limitations, but this problem is beyond this technical note. Also sufficient air humidity is important to prevent plants from loosing excessive amounts of water (Karlberg et al., 2006). Thin structures like leaves can dry out even when water supply from the roots via the vascular system is not limiting. Given a certain amount of water vapor in the air, the upper limit of air humidity is determined by the so called dew point temperature. This is the temperature at which condensation forms on the leaves. This promotes fungi like Botrytis to grow (http://www.redpathaghort.com/bulletins/ControllingHighHumidity). It also can prevent plants from sufficient transpiration, which is required for adequate mineral supply from the roots to the remainder of the plant (http://www.redpathaghort.com/bulletins/ControllingHighHumidity) (Spomer and Tibbitts 1997). The optimal setting points are 60 to 70% relative humidity (RH) Not much data is available on actual crop losses if optimal humidity range is not maintained. Considerable damage is only to be expected in combination with additional factors like the presence of pathogens in case of too high humidity.

## 2.5. Nutrients

The nutrient requirement for crops in general is well studied. For individual crops available hydroponic nutrient solutions might still be optimized but good yields will be possible with present standard nutrient solutions. The required major elements are: nitrogen, phosphorous, potassium, calcium, magnesium and sulfur, and the minor elements are iron, boron,

manganese, copper, zinc, chloride and molybdenum. At the Crop Physiology Laboratory at Utah State University optimized nutrient solutions for monocotyledonous and dicotyledonous crops were developed (http://www.usu.edu/cpl/research_hydroponics.htm). The required amounts of these solutions vary with the developmental stage of the respective crops. The developmental periods of different nutrient requirements are defined as starter, pre-anthesis and post-anthesis. The total amount of nutrients required is different for each crop. Administration/refilling of can be controlled by EC values (electric conductivity) which indicate the total amount of nutrition salts; this is considered to be a rather reliable parameter.
In commercial greenhouse crop growing this parameter is used in combination with pH measurements to monitor the nutrient solution, pH is accepted to be optimal between 4.5 and 5.5 for a wide range of crops, which include the MELiSSA crops.
Optimal EC value ranges for some MEliSSA crops are indicated in table 1. Tomato fruit yield at EC values 1.5, 3.0, or 4.5 did not vary significantly in a research experiment done in the Research Center Hoogstraten, Belgium (Year report 2004). The researchers did however mention that, at high EC values the plants were more sensitive for physiological disorders like nose rot (malformation of the fruit tip) and pathogens. This enhanced sensitivity was attributed to unbalanced ion uptake. For instance, calcium is a slowly consumed and transported mineral that is negatively discriminated when total ion concentration is too high.

**Table 1**

| MELiSSA Crops | Optimum range EC (mS/cm) | Ref. |
|---|---|---|
| Lettuce | 0.3-1.2 | http://www.hydrosupply.com/NewFiles/About.html |
| Beet | 1.8-2.2 | http://www.hydrosupply.com/NewFiles/About.html |
| Onion | 1.8-2.2 | http://www.hydrosupply.com/NewFiles/About.html |
| Spinach | 1.8-2.3 | http://www.hydrosupply.com/NewFiles/About.html |
| Tomato | 2.2-2.4 | http://www.hydrosupply.com/NewFiles/About.html |
| Potato | 0.12 | (Wheeler, 2006) |
| Soybean | - | - |
| Wheat | - | - |
| Rice | - | - |

The essential nutrients can be separated into three categories based on how quickly they are removed from the solution (Table 2). The ions from group one are actively taken up, and if monitored in the solution the concentration will be low. This indicates healthy plants rather than lack of nutrient. If concentrations are individually adjusted based on concentration in the recirculated nutrient solution their concentration must be kept low to prevent toxic accumulation. Low concentrations are however difficult to monitor and control. Therefore it is better to control the optimal composition of the solution based on desired concentration of each element in the plant. These values should be derived from reference values from healthy, high yielding crops (http://www.usu.edu/cpl/research_hydroponics3.htm).

**Table 2** Approximate uptake rates of the essential plant nutrients

| | | |
|---|---|---|
| Group 1 | Active uptake, fast removal | $NO_3$, $NH_4$, P, K, Mn |
| Group 2 | Intermediate uptake | Mg, S, Fe, Zn, Cu, Mo, C |
| Group 3 | Passive uptake, slow removal | Ca, B |

The Belgian Research Center St-Katelijne-Waver used one standard solution for the tomato culture. Based on bi-to tri weekly measurements of ions in recycled solution, individual ions were adjusted. This might be justified for greenhouse cultures, since environmental parameters and thus growth can vary dramatically, for example, from very low radiation on cloudy days to very high radiation on sunny days, but it might also lead to the above sketched problem of group one ion overdosing and consequently lack of ions from group two or three. However in closed culture, environmental conditions should be very stable and nutrient refill solutions are then best standardized based on the developmental stage of the crop only (by using the determined optimal nutrient contents as a reference for each of the chosen crops).

Nutrient supply has not been considered a critical input in previous MELiSSA HPC projects, because it was assumed optimal in the case of hydroponics (Cloutier and Dixon, 1998). However, this has not always been justified since the growth rate for lettuce empirically determined by them was 1.7 g DW $m^{-2}$ $d^{-1}$, which is far from optimal as shown by Frantz *et al.* (1994), who reported 19 g DW $m^{-2}$ $d^{-1}$ as maximum growth rate averaged over the total growing period of 23 days. This less than optimal growth is due to the choice of conditions that prevent tip burn (physiological disorder caused by insufficient calcium transport whereby leaf edges and growing tips get necrotic, and also new leaf emergence is hampered) by reducing light intensity and temperature. This solves the problem of tip burn but also reduces yield. The true limiting factor underlying tip burn is lack of calcium in the affected plant parts. Franz *et al.* (1994) solved this problem by increasing the ventilation in the growth chamber; especially direct ventilation on the leaf tips is effective. Because calcium is slowly transported in the plant a high transpiration rate (flow of water through the plant) is necessary to supply the more peripheral plant parts with this essential element. This physiological disorder tip burn in lettuce, was also mentioned by the Belgium Research Centers as a main problem. Besides reducing yields it can render the plants also more susceptible to opportunistic pathogens such as Botrytis. In addition, nose rot, or blossom end rot, in tomato (physiological disorder causing rotting of tomato at the distal end, also due to calcium lack in the affected tissue) was also mentioned as common problem by the Belgium Research Centers. Summarizing we can conclude that lack of calcium is a commonly occurs in lettuce and tomato crop cultures (maybe in dicotyls in general), leading to physiological damage and reducing yield. It is due to calcium deficiency in the affected distal plant parts, caused directly (by lack of calcium in the nutrient solution) or indirectly (by unbalanced nutrient solutions or insufficient transpiration).

Also lack or excess of nutrients are well described for many crops. An overview of mineral deficiencies in several crops (including the MEliSSA crops: lettuce, potato, spinach, table beet and wheat) and the corresponding visual symptoms can be found at http://www.hbci.com/~wenonah/min-def/list.htm.

## 3. Weeds, pests and pathogens

With respect to MELISSA crops, the loss potential and the actual losses-i.e. losses despite the present crop protection practices- have been estimated for wheat, rice, potatoes and soybeans for the period 1996-1998 by Oerke *et al*. (2004). Among these crops the loss potential due to pests worldwide varied from less than 50% to more than 80%. Actual losses are estimated to be 26-30% for wheat and soybean and about 40% for potatoes and rice. Overall, weeds had the highest loss potential (32%) with animal pests and pathogens being less important (18% and 15% respectively).

While weeds have the highest loss potential for soil grown crops, hydroponic culture eliminates this problem altogether. Also animal pests should not be a problem in closed culture systems if sterilized starter material and clean working procedures are followed. Plant pathogens, including fungi, bacteria and viruses are more prone to be present in space-based plant growth facilities.
Viruses cause little damage globally but can cause large problems in some region for some crops, e.g. potatoes. Good cultural practice reduces the incidence of virus infections but in case of an infection, besides removing infected parts, no measures can be taken against it during the growth season. Sanitation and a clean new start (virus free starting material, seeds or seedlings) will solve the problem for a next crop cycle. Use of virus resistant cultivars is an additional effective means against virus occurrence. Also in hydroponic systems viruses could be a potential problem and again, good cultural practice (prevention) will be the key to eliminate or reduce the problem. Elimination of the problem should be possible because the culture takes place in a closed environment and the culture space can be sterilized. This will not only remove the virus itself but also the hosts if it needs an intermediate to be spread from plant to plant (e.g. insects). The following measures should be taken to prevent occurrence of viruses:

- Use resistant cultivars if available.
- Sterilize growth rooms and restrict access.
- Use clean starting material. Sterilize starting material.
- Maintain optimal plant distance. Too high densities cause local spots of high/excessive humidity and enhanced risk for virus infections.
- Control humidity at optimal level and provide sufficient airflow.
- If pruning or cutting is necessary do it in the morning so that wounds can dry out in time, in a completely controlled environment humidity could also be temporarily lowered.

Besides viruses, many plant pathogenic fungi, cause crop losses. In agriculture pesticides are commonly used to control fungi populations and reduce losses. In greenhouses complete losses of crops are expected if no treatment would take place, due to often ideal conditions for fungal

growth. In advanced life support systems these pathogens might therefore cause major problems. Prevention is wanted but might be hard to achieve. In the Belgian research centers three fungi were mentioned as regularly returning major problem pathogens for lettuce as well as tomato: *Botrytis*, *Pythium* and *Sclerotinia*.
In the research centers visited (St-Katelijne-Waver and Hoogstraten), Botrytis was mentioned as one of the main problem pathogens for horticulture in lettuce as well as tomato. The host range of Botrytis stretches even further among the MELiSSA crops.
Major hosts for Botrytis (grey rot) of the listed MELiSSA crops are (CABI Crop Protection Compendium; http://www.cabicompendium.org/cpc/home.asp):

Allium cepa (onion)
Lactuca sativa (lettuce)
Lycopersicon esculentem (tomato)

Minor hosts for Botrytis of the listed MELiSSA crops are (CABI Crop Protection Compendium; http://www.cabicompendium.org/cpc/home.asp):

Glycine max (soybean)
Triticum aestivum (wheat)

Solanum tuberosum (potato) is a host for Botrytis of which the status of possible losses is unknown (CABI Crop Protection Compendium; http://www.cabicompendium.org/cpc/home.asp):

In the literature Botrytis is sometimes mentioned as causing severe damage. Specific data on the severity of damage caused by Botrytis under given conditions is however not available. Rudi Aerts (Katholieke Hogeschool Kempen, Dept. Industrial sciences and Biotechnique, Geel, Belgium), an expert on Botrytis reported that such data most likely can not be found in publications, which confirms our failure in finding quantitative data. In recent years these researchers have been counting infections on tomato at several horticultural companies during a whole year. The numbers they found varied a lot. At one company with high infection pressure an average of one infection per plant per season was recorded. In case no chemical treatment would take place all plants would die from Botrytis. This is prevented by chemical treatment and by allowing extra branching. But even though treatments take place a loss of 10 to 20% is possible. Well-run companies have losses in the order of 1 to 5% due to Botrytis (Rudi Aerts, personal communication). Since pathogen infections, like Botrytis, can be totally devastating without chemical treatment to a crop like tomato, it can induce a severe problem in closed culture systems where chemical treatment is most likely excluded/not wanted due to recycling issues.
A second major pathogen mentioned by the Belgian research centers Beitem, Hoogstraten and St-Katelijne-Waver, also regarding lettuce as well as tomato, was Pythium, which causes root rot. The presence of this pathogen has even increased specifically due to increased popularity of hydroponic culture systems. This pathogen produces zoospores and the production and spreading thereof is favoured by abundant water supply (Herrero *et al*., 2003). *Pythium spp*. cause damping-off of seedlings in vegetables and may kill feeder roots but are seldom able to kill more mature plants (Herrero *et al*., 2003).

The research centres had in general no problem with bacteria in lettuce or tomato. Only one occurrence of Corynebacteria (Clavibacter michiganensis, which colonizes the plant vascular system and ultimately causes external plant cancers, http://www.umass.edu/umext/programs/agro/vegsmfr/Articles/Disease/v2.htm) was reported. Chemical treatment was also used in this case to control the problem.

Four viral, three bacterial and 21 fungal pathogens have been identified as causal agents of root disease in hydroponically-grown crops, although originally avoidance of root-infecting (typically soil-borne) microorganisms was considered one of the advantages of hydroponically-grown crops (Stanghellini and Rasmussen, 1994).

There are some reports showing that plants have increased susceptibility to fungal pathogens when grown in migrogravity (Leach *et al.* 2001; Ryba-White *et al*., 2001). This is one more reason to work towards prevention of contamination rather than corrective treatment. There are reports giving numbers of pathogens (fungi as well as bacteria) present during space flights or at the International Space Station. These numbers were even higher for the space shuttle during flight (Pierson, 2001). However, none of these reports distinguished plant pathogens specifically, making it still difficult to access the actual risk.

There are several technologies available to reduce the prevalence of airborne pathogens. Two known pathogen-killing techniques are photocatalytic oxidation (PCO) and ultraviolet (UV) light treatment. According to the Belgium research centres Hoogstraten and St-Katelijne-Waver, UVC radiation was used as a standard measure to treat recycled nutrient solutions. The patented technology used in AiroCide™ combines both technologies to destroy harmful airborne microbes (http://www.kesair.com/). Titanium dioxide (TiO2) is the photocatalyst used in the AiroCide product. When this material is irradiated with ultraviolet light, strong oxidizing agents (hydroxyl radicals and super-oxide ions) are formed. These agents enable AiroCide to kill/remove/eliminate airborne pathogenic and non-pathogenic microorganisms in vegetative and spore states (bacteria, fungi, viruses and dust mites), as well as, allergens, odours and (harmful) volatile organic compounds (VOC's ). For the latter mentioned compounds it is also an interesting technology to maintain low levels of e.g. harmful allelopathic compounds like ethylene (see also next section). Because the organic material is completely oxidized by this process, the photocatalytic reactor is self-cleaning and no organic material accumulates on the catalyst surface. In this process no ozone is generated, the end products are water vapour and carbon dioxide. Several case studies showed the effectiveness of the technology to reduce airborne pathogens. In a tomato cooled storage room fungi were reduced by 54% in 24 hours and 62% in 48 hours with the AiroCide, while bacteria in the same cooler were reduced by 75% in 24 hours and 100% in 48 hours.

# 4. Allelopathic factors

Closed loop plant growth has its specific advantages, but it also has its unique problems. One of the major problems specific for cultures in closed environments is accumulation of plant excretion products which can influence their own growth, or the growth of other crops integrated in the loop. Ethylene is the best known example and of major importance. It is a gas with hormonal properties (De Paepe and Van Der Straeten, 2005). In the fields it rarely accumulates to levels with unwanted effects due to quick dissipation, but in closed systems however, it quickly rises to levels with inhibiting effects. Ethylene is an endogenously synthesized plant hormone seldom exceeding 1-5 ppb in the field but accumulating 10-100 times in controlled environments. The effects of high ethylene levels are dependent on the crop but include reduced growth rate, senescence, sterility and too fast ripening. Ethylene levels on the International Space Station have been maintained at 50 ppb, but several studies have clearly indicated that plant growth and yield are significantly reduced by levels as low as 20 ppb. Since plants themselves produce ethylene, it is extremely difficult to maintain atmospheric levels below 20 ppb. (Campbell *et al*., 2001; Klassen and Bugbee, 2002; Klassen and Bugbee, 2004)

Ethylene sensitivity varies not only between crops but also between varieties of a crop. Yield of USU-Apogee wheat was not significantly reduced by 50 ppb ethylene but the yield of Super Dwarf wheat was reduced by 60% at that low level of ethylene (Klassen and Bugbee, 2002). Therefore care needs to be taken by the choice of a cultivar and extrapolation of data should not be done automatically, i.e. empirical testing for individual varieties will be necessary. In the same study a difference in the ethylene sensitivity of USU-Apogee between greenhouse and growth chamber trials also suggested that ethylene sensitivity is dependent on the environment. This was confirmed in studies on tomatoes where <u>temperature</u> was shown to have a large effect on ethylene sensitivity. Ethylene sensitivity decreased with increasing temperature and more so for the cultivar Red Robin than for Micro-Tina. At 22 $^{o}$C, Red Robin yield with 30 ppb ethylene treatment was about 42% less than control with no apparent decrease at 28 $^{o}$C. Micro-Tina yield with 30 ppb ethylene treatment decreased by about 22% at 22 $^{o}$C with no apparent decrease at 28 $^{o}$C. Red Robin yield was 20 to 60% less than Micro-Tina in all treatments. Both cultivars had 40 to 60% higher yield at 22 than at 28 $^{o}$C. (Bugbee and Klassen, 2004)

Not only ethylene sensitivity varies from crop to crop and from variety to variety, but also ethylene production. Ethylene sensitivity as well as production also depends on the developmental stage of a crop (Smalle and Van Der Straeten, 1997 and references therein). Therefore it is clear that in a closed culture with more than one crop they can negatively influence each other through the effects of ethylene. Controlling ethylene levels is therefore of major importance. Maintaining ethylene levels low can be performed by combined photocatalytic oxidation (PCO) and ultraviolet (UV) light technologies as mentioned in the previous section. The efficacy of the method for removing ethylene is not clear from published data. Ethylene also could be removed by scrubbing technologies. Potassium permanganate ($KMnO_4$, Purafil) binds ethylene and is far superior to UV-catalyzed degradation, but the air flow rates and quantity that are needed to keep ethylene below 10 ppb are extremely expensive (Plant Physiology Laboratory, USU, http://www.usu.edu/cpl/research_ethylene.htm).

Besides ethylene there are other allelochemicals from which effects on crops are much less known, but from which especially in repeated cycles of closed crop cultures detrimental effects can be expected. These include phenolic acids, which can be excreted by plant roots (Pramanik *et al*., 2000). Phenolic acid treatments of cucumber seedlings (Cucumis sativus cv "Early Green Cluster") inhibited transpiration, water utilization, leaf area, and absolute and relative rates of leaf expansion (Blum and Gerig, 2005). The cinnamic acids, ferulic and p-coumaric acid, were two to five times more inhibitory than the benzoic acids, p-hydroxybenzoic acid and vanillic acid. Lettuce seed germination is inhibited at 1 mM p-coumaric acid (Rimando *et al*., 2001). A lettuce seedling bioassay showed no evidence of allelopathic compound accumulation in an intercropped recirculating hydroponic system of wheat and tomato (Schuerger and Laible, 1994). At present, efforts are made to breed allelopathic cultivars in several crops (Belz and Hurle, 2004; Kong et al., 2004; Ma, 2005) . These are crops that control weeds by root exudation of allelochemicals. For advanced life support systems it is probably sensible to do the opposite and select for the least allelopathic cultivars of all the crops used in the system to prevent these crops from negatively influencing each other by these allelochemicals in a recirculating system.

# 5. Space related stress factors

Research information on the effects of a lower total pressure on plant development is not yet available.

Sufficient airflow and mixing around the plant leaves is a critical issue that needs to be addressed to ensure optimal productivity by allowing maximal $CO_2$ influx into the leaf (Porterfield 2002;. Kitaya et al., 2003; Monje et al., 2005).

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